Kerstin Svahn

Coccidian Blood Parasites in Lacertids

Lund 1972

COCCIDIAN BLOOD PARASITES IN LACERTIDS

AKADEMISK AVHANDLING

för doktorsexamen som med vederbörligt tillstånd av matematisk-naturvetenskapliga fakulteten i Lund kommer att offentligen försvaras å Ekologihuset, Helgonavägen 5, Lund, onsdagen den 17 maj 1972 kl 9.00.

av

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Printed in Sweden
Studentlitteratur
Lund 1972

The thesis comprises the following papers:

I. Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards. Description and Life Cycle. MS 32 pp. (1972).

Blood films from lizards of the genus Lacerta from southern Sweden and Denmark were studied in order to examine the species of blood parasites of haemogregarine type. The parasites found were Karyolysus lacazei Labbé, 1894 in L. agilis L. and L. vivipara Jacquin, K. lacertae Danilewsky, 1886 from L. agilis, K. latus sp. nov. from L. agilis and L. vivipara and K. minor sp. nov. from L. agilis.

As haemogregarine parasites cannot be classified until their life cycle is known the study has been concerned with the development of the parasites. Successful transmission of Karyolysus had previously been achieved by feeding lizards with mites experimentally invaded by the parasite. The mite Sauronyssus saurarum Oudemans was reared in laboratory and found to be the transmitter. The parasites became free in female mites. After fertilization an oocyst containing motile sporokinetes developed. The sporokinetes found their way to the eggs where they encysted as sporocysts. Karyolysus was transmitted when the infected nymphs were eaten by a lizard. The prepatent period in lizards ranged from 30 to 37 days.

II. Incidence of Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards. MS 25 pp. (1972).

Karyolysus latus was the most common parasite in lizards in Scania. It was found only in one locality in Denmark. The most spread parasite in Denmark was K. lacazei which also occurred in Småland, Sweden. K. lacertae was found only in one locality in Denmark and K. minor both in Scania and Denmark.

The size measurements of K. latus from different localities were statistically treated. The differences between mean values of the sizes of macrogametocytes were not significant.

From a study of seasonal occurrence of Karyolysus it appeared that spring was the most favourable season for transmission. The great number of mites found in spring, the renewed infections in autumn, and the hibernation of the infection in lizards confirmed this opinion. The infection acquired in spring and recorded in autumn will disappear next summer.

Lizards developed some resistance. Thus the heaviest infections of K. latus were developed in juvenile animals.

III. A new piroplasm Sauroplasma boreale sp. nov. from the Sand Lizard Lacerta agilis L. MS 7 pp. (1972).

Lizards from southern Sweden and Denmark were examined for piroplasms. A new species Sauroplasma boreale was described from a locality at the Lake Snogeholmssjön in Scania and from Mön in Denmark. The genus Sauroplasma du Toit, 1937 was described from South Africa. The parasites invaded the erythrocytes and formed a ring with a marked outer membrane. No granules or pigment could be detected in the cytoplasm. The nucleus almost or completely surrounded the clear part of the cytoplasm. The smallest forms were anaplasmoid bodies 0.8 µm in diameter, the largest form measured 3.5 to 3.8 µm.

Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards. Description and Life Cycle

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CONTENTS

Summary of thesis 3

I Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards. Description and Life Cycle 5

Abstract 9
Introduction 10
Materials and methods 11
The arthropod 12
Natural incidence of Karyolysus in Scandinavian lizards 12
Descriptions 13
1. Karyolysus lacazei Labbé 13
2. Karyolysus lacertae Danilewsky 15
3. Karyolysus latus sp. nov. 16
4. Karyolysus minor sp. nov. 19
Developmental stages of Karyolysus in mites 20
Development and pathology of Karyolysus in lizards 24
References 26
Tables 1-2 28
Figures 1-34 30

II Incidence of Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards 57

Abstract 59
Introduction 60
Materials and methods 60
Study area and host materials 61
Incidence of Karyolysus in Swedish and Danish populations of Lacerta 62
Variation of gametocyte size 64
Seasonal incidence of Karyolysus in southern Scandinavian Lacertae 65
The course of infection in Lacertae 68
References 71
Tables 1-7 72
Figures 1-2 83

III A new Piroplasm Sauroplasma boreale sp. nov. (Protozoa, Haemosporidia) from the Sand Lizard Lacerta agilis L. 87

Abstract 89
Introduction 89
Materials and methods 89
Description of Sauroplasma boreale sp. nov. 90
Development and pathology of S. boreale in lizards 91
References 93
Figures 1-16 94

ABSTRACT

Svahn Kerstin. 1972. Blood Parasites of the Genus Karyolysus (Coccidia: Adeleidae) in Scandinavian Lizards. Description and Life Cycle. Four species of Karyolysus Labbé, 1894 from the lizards Lacerta agilis L. and L. vivipara Jacquin were found in southern Sweden and Denmark. Two new species are described. Of 159 L. agilis and 37 L. vivipara 24 L. agilis and one L. vivipara were parasitized by K. latus sp. nov., 38 L. agilis and 3 L. vivipara by K. lacazei Labbé, 2 L. agilis by K. lacertae Danilewsky and 3 L. agilis by K. minor sp. nov. Karyolysus developed successfully in the mite Sauronyssus saurarum Oudemans, reared in laboratory. In the female mite, the zygote grew to an oocyst which contained motile sporokinetes that found their way to the eggs where they encysted as sporocysts. Karyolysus was transmitted when the infected nymphs were eaten by an uninfected lizard. The prepatent period in the lizards ranged from 30 to 37 days. Biologically and morphologically the life cycle is similar to that of other Karyolysus species as far as described.

INDEX KEY WORDS: Adeleidae; Coccidia; Denmark; description; Karyolysus latus; K. lacazei; K. lacertae; K. minor; Lacerta agilis; L. vivipara; life cycle; new species; Sauronyssus saurarum; Sweden; transmission.

Since Danilewsky (1886) described the first Haemoprotozoa in the blood of Lacerta viridis Laurenti and L. agilis L. from Kharkoff in Ukraine many descriptions of saurian blood parasites have been published. Numerous species have been named, especially of the genus Haemogregarina Danilewsky, but the old descriptions are often so incomplete that the species are impossible to identify. In some cases different species of parasites have been named collectively. Thus Danilewsky when describing Haemogregarina lacertae from L. viridis and L. agilis was obviously dealing with two species. In other cases confusion has been caused by the assumption that a parasite found in a new host must necessarily be a new species. França (1909, 1912) apparently described young gametocytes as separate species. Thus there is a large number of nominal species of Haemoprotozoa in the literature. Only in a few cases the life cycle and the intermediate host have been described, e.g. for the genera Haemogregarina and Karyolysus Labbé by Reichenow (1910, 1913, 1921).

Most records of blood parasites in Lacerta concern south Europe, France and north Africa. Labbé (1894) described Danilewskya lacazei and K. lacertarum from L. agilis. L. muralis Laurenti and L. lepida Daudin (L. ocellata Daudin) from France. França (1909, 1912) described many species of Haemogregarina in L. ocellata and L. muralis from Portugal. Georgevitch (1940) described Haemogregarina mellisselensis in L. serpa Rof. var. Galvagnii Werner from islands in the Adriatic.

No blood parasites in lizards were recorded from north Europe until Markov (1950) when studying L. vivipara Jacquin in the Leningrad region found a parasite which he identified as Karyolysus lacertae Danilewsky.

There are no previous records of Haemoprotozoa from Scandinavian reptiles. The first Haemoprotozoa found was a haemogregarine parasite in a blood film from the lizard L. agilis, collected from Scania, southern Sweden. In searching for similar parasites lizards from 29 localities in southern Sweden and Denmark were investigated. Four species of the genus Karyolysus were found and two new species are described in the present paper. As parasites of the haemogregarine type cannot be correctly classified until their life cycle is known this study has been concerned with the development of the parasites.

Attempts to transmit Karyolysus from infected L. agilis to larvae and nymphs of the tick Ixodes ricinus L. were unsuccessful. Instead the mite Sauronyssus saurarum Oudemans was found to be the transmitter.

MATERIALS AND METHODS

Mites were collected from the lizards as follows. After capture the lizards were placed in a glass vessel standing in water. In the morning mites had left the lizards and crept on the vessel. They were collected with a brush and kept in test-tubes with damp filter-paper. The tubes were stored at temperatures ranging from 21-30° C.

The infection in the mites was studied by dissecting specimens at hourly intervals for the first day's postinfection and thereafter every day. The material was examined in physiological saline or as permanent films or sections. Permanent films of dissected material and blood films from the tail-tip were dried in air, fixed in methanol and stained by Giemsa's method. Mites to be sectioned were treated after puncture with Helly's fixative or with Bouin-Hollande sublimate and 4-8 μm sections were cut according to the usual paraffin method. After embedding Westopal W 3 μm sections were cut on an LKB ultrotome 8800. Internal organs of lizards were fixed in Helly's solution and 5-8 μm sections were prepared. Several stains were used, of which the best proved to be 1) Ehrlich's haematoxylin-eosin, 2) Delafield's haemotoxylin, 3) Delafield's haematoxylin-Orange G-Light Green, 4) the Azan-method of Heidenhain, 5) PAS (Periodic acid-Schiff reaction), 6) Gomori's chrome alun haematoxylin (Romeis 1968, Pearse 1960).

As the sclerotized integument of hungry nymphs would not be so easily destroyed in the intestine of the lizard, the nymphs were given the lizards well-fed, pasted on meal-worms.

During the period when - according to experience - parasitemia would first occur, blood films were prepared at intervals of 1-3 days from animals which had been fed infected mites. Thus the prepatent period of Karyolysus in the vertebrate host was determined.

THE ARTHROPOD

Below certain details on the biology of Sauronyssus saurarum are presented as a background to the description of the sporogonic cycle of Karyolysus. The morphology of the mite was described by Oudemans (1901).

One day after hatching the six legged larvae moult and become nymphs with eight legs. Some days later the nymphs attack the lizards to suck blood. When they leave their hosts 2-4 days later the male and female nymphs can easily be distinguished, the females being larger than the males. After feeding, the nymphs have a red colour and they usually leave the lizards paired. The female nymphs carry the males on their backs when seeking for a hiding-place. The time required for the development of nymphs into sexually mites was estimated by Reichenow (1921) to be about 24 hours. It is unknown if adult males feed. After some days the sexually-mature females attack the lizards for their first blood-meal. On blood-sucking they become larger and spherical. Mites placed on lizards in the evening fall from their hosts the next morning, when they seek a hiding-place for oviposition. After 1-2 days the first eggs may be observed and about 4 days later the larvae hatch out. About 6-8 days after feeding the females attack a lizard for a second blood-meal, and after 10-12 days, a third. Each meal is followed by oviposition. The number of eggs layed ranges from 10 to 20 and depends on the quantity of blood ingested. Oviposition took place at 21-30° C. At lower temperatures the development was prolonged.

NATURAL INCIDENCE OF KARYOLYSUS IN SCANDINAVIAN LIZARDS

Of 159 Lacerta agilis L. and 37 L. vivipara Jacquin collected from southern Sweden and Denmark between July 1949 and September 1970, 24 L. agilis and one L. vivipara were parasitized by K. latus sp. nov., 38 L. agilis and 3 L. vivipara by K. lacazei Labbé, 2 L. agilis by K. lacertae Danilewsky and 3 L. agilis by K. minor sp. nov. Multiple infections (maximum two species) were found in 4 lizards.

DESCRIPTIONS

Genus Karyolysus Labbé, 1894

The gametocytes of the genus Karyolysus live in blood corpuscles, the nuclei of which are usually displaced, sometimes compressed, sometimes swollen, and more faintly staining than those of a normal blood-cell. The host cell hypertrophies and the cytoplasm - or what remains of it - loses its staining properties and is difficult to observe in the preparations. In most cases it completely disappears. Sometimes dark purple granules occur in the gametocytes, but they have nothing to do with genuine pigment.

1. Karyolysus lacazei Labbé, 1894

Hosts: Lacerta agilis L., L. vivipara Jacquin.
Localities: L. agilis (2 specimens) from Skillingaryd, Småland, 1960; L. agilis (11 specimens) from Tein, Bornholm, 1960, 1961, 1962; L. agilis (13 specimens) from Melsted, Bornholm, 1965; L. agilis (5 specimens) from a locality west of Vordingborg, Zealand, 1967; L. agilis (1 specimen) from Svanninge, Funen, 1966; L. agilis (6 specimens) from Anholt, 1969; L. vivipara (3 specimens) from Melsted, Bornholm, 1964, 1965.
Gametocytes: The gametocytes have a slender, wormlike shape and are bent. One end, where the nucleus is often situated, is thickened, the other end is thin and often pointed. The cytoplasm of the gametocytes stains pale blue or pinkish with Giemsa's stain and sometimes, therefore, can be recognized only with difficulty in the blood films. Sometimes a few red granules can be noted. There are no vacuoles in the cytoplasm of adult gametocytes. Measurements of 200 gametocytes of K. lacazei from 11 L. agilis were 17.7-27.7 μm (mean 22.0 μm). Maximum breadth of 75 gametocytes from 5 L. agilis was 2.7-4.6 μm (mean 3.6 μm). The nucleus is elongate, sometimes well defined, sometimes more diffuse. It stains bright red. Measurements of 25 nuclei of K. lacazei from 2 L. agilis were 3.8-8.1 μm × 1.9-3.8 μm (mean 5.4 × 2.9 μm). No nucleolus has been observed. The location of the nucleus in the gametocytes is variable (Fig 1). It has not been possible to distinguish macro- and microgametocytes in blood films. Gametocytes moving freely in the vector can easily be distinguished as macro- and microgametocytes respectively.

Trophozoites: The shape varies from slender, crescentic to broader, reniform. Measurements of 25 trophozoites were 11.9-17.7 μm × 1.9-5.4 μm (mean 14.3 × 3.9 μm). The cytoplasm has some sharply defined vacuoles and the large granular or reticular nucleus is usually centrally placed (Figs 2-3). Round, vacuolated parasites are obviously trophozoites.

Effects of the parasite on the host cell: The presence of the parasite causes very great changes in the appearance of the host cell. It hypertrophies and the cytoplasm, or what remains of it, loses almost completely its staining properties and is observed only with difficulty in the preparations. In most cases it is completely absent. The nuclei of infected cells are usually swollen and have a paler staining reaction than those of uninfected cells. Sometimes the host cell nucleus is compressed and darkly stained. The nucleus is displaced already at an early stage of the infection.

Schizonts: Only one kind of schizonts was found in sections of liver, lung, heart, ovary and spleen. In one case a schizont was found in the intestine. The schizonts were studied in serial sections and found to be ovoid bodies. Twenty schizonts measured 11.5-30.8 μm × 7.7-18.5 μm (mean 19.9 × 12.1 μm). Young schizonts showed an undifferentiated central mass, with nuclei arranged peripherally (Fig 4). In the most advanced stages the centre of the schizonts was filled with slender elongate merozoites, each about 8-12 μm, with very dense rather oblong nuclei (Fig 5). It was difficult to count the merozoites but in most cases 16-32 were found. Sometimes a rest body could be observed.

General: The infection is often very heavy. Occasionally two or more parasites are present in a single cell (Fig 3).

Discussion: In the paper of Labbé (1894) the name Danilewskya lacazei is used for a parasite found in Lacerta agilis and L. muralis. Labbé suggests that it may be the species described by Danilewsky in 1886 as young stages under the name of Haemocytozoon clavatum from L. viridis and L. agilis at Kharkoff. The species has also been recorded by Reichenow (1921) from L. muralis in Spain. Apparently (cf. Reichenow 1921) gametocytes of this species were regarded by Woodcock (1912) as schizonts. The parasite found in Scandinavian L. agilis and L. vivipara agrees well with the description of Karyolysus lacazei given by Labbé. In the Scandinavian material the length of the parasite varied from 18 to 28 μm, which agrees fairly well with the data of Labbé (25-28 μm) and Reichenow (25-32 μm). Reichenow (1921) illustrated macro- and microgametocytes of K. lacazei with both ends at the same side of the host cell nucleus. I have found that the vermicules more often curve round

the periphery of the blood cell, almost completely surrounding its nucleus, or in other cases are doubled up and push the nucleus to one end of the cell. Labbé found only one kind of schizonts in bone-marrow and spleen. The schizonts measured 15-20 μm in legnth and contained 15-20 merozoites and thus agreed with those found in Scandinavian lizards.

2. Karyolysus lacertae Danilewsky, 1886

Host: Lacerta agilis L.
Locality: The following description is made on parasites found in the blood of two L. agilis from Tejn, Bornholm, 1962.
Gametocytes: The gametocytes have rounded ends and are slightly bent. They are surrounded by a capsule which prevents the stain from penetrating into them (Fig 6). Thus the cytoplasm stains very faintly blue or is colourless and sometimes no nucleus is visible. Sometimes a chromophil cap is visible at the end of the parasite opposite the nucleus (Figs 6-7). Fifty-three gametocytes from 3 L. agilis measured 12.3-14.6 μm x 3.9-4.6 μm (mean 13.6 x 4.3 μm). The nucleus is reticular, red and situated at one end of the parasite. The mean of 20 nuclei was 3.7 x 3.0 μm. It has not been possible to distinguish macro- and microgametocytes.
Trophozoites: The trophozoites have a broad, ovoid shape and measured 12-13 μm x 5 μm. Their cytoplasm is blue and vacuolated and the nucleus is centrally placed. No capsule has been detected around them (Fig 8).

Effects of the parasite on the host cell. Gametocytes cause hypertrophy of their host cells. Such infected cells stain paler than uninfected ones, the nucleus being swollen or sometimes compressed and strongly displaced.

Schizonts: Two kinds of schizonts were present in organs of infected lizards. There were round and oval schizonts producing numerous slender micromerozoites and ovoid schizonts producing relatively few, large macromerozoites. Reichenow (1913, 1921) interpreted the schizonts with large merozoites as asexual and giving rise to new schizonts, and the schizonts with numerous small schizonts as sexual, giving rise to numerous merozoites, which penetrate red cells and become gametocytes.

Numerous sexual schizonts with small nuclei were found in sections of lung, liver, heart, spleen, ovary and in smears from the tail tip. They

were round or ovoid bodies containing about 32 merozoites (Figs 9-10). Twenty schizonts measured 11.5-20.0 μm × 9.2-13.8 μm (mean 14.8 × 11.5 μm). The merozoites were about 9 μm with round dense nuclei. Two schizonts from liver smears measured 26.9 × 17.7 μm and 29.2 × 19.2 μm (Fig 10).

Asexual schizonts were found in sections of lung and spleen. They were ovoid and the measurements of 15 specimens were 13.1-17.7 μm × 8.5-13.1 μm (mean 15.7 × 11.4 μm). Usually 8-12 merozoites were visible in a longitudinal section (Fig 11). The merozoites were large, about 10 by 3 μm, with large, dense nuclei. Unfortunately, the lizards investigated had a mixed infection with K. lacazei, so that it was impossible to say to which species the youngest schizonts belonged.

General: Infections of K. lacertae are often very heavy in laboratory conditions. Blood cells with double infections were often observed.

Discussion: This species was described by Danilewsky (1886) as Haematozoa lacertae from Lacerta viridis and L. agilis from Kharkoff, but his material evidently contained two species, which were probably K. lacertae and K. lacazei. The empty capsules of K. lacertae in lateral view are hour-glass shaped and Danilewsky has an illustration of such an empty capsule in his description. The name was fixed to the present species by Reichenow (1913).

Labbé (1894) described K. lacertae as a new species under the name of K. lacertarum. He identified K. lacertarum with the parasite illustrated by Danilewsky. The same species was probably studied by Marceau (1901) and Markov (1950, 1952).

The life history of K. lacertae has been worked out by Reichenow (1913) who showed that schizogony occurred in the lungs, liver and spleen. He also found schizonts in blood from the tail-tip. Two kinds of schizonts were found and the number of merozoites was sometimes more than one hundred. The parasite studied by Reichenow agrees well with the one found in Scandinavia.

3. Karyolysus latus sp. nov.

Hosts: Lacerta agilis L., L. vivipara Jacquin.
Localities: L. agilis (6 specimens) from Snogeholm, Scania, 1949,

1957-1960, 1962; L. agilis (12 specimens) from Maglehem, Scania, 1962, 1964-1967, 1969; L. agilis (3 specimens) and L. vivipara (1 specimen) from Degeberga, Scania, 1967, 1968; L. agilis (1 specimen) from Hallamölla, Scania, 1968; L. agilis (2 specimens) from Brösarp, Scania, 1963, 1969; L. agilis (1 specimen) from Gilleleje, Denmark, 1967.

Type: Slide from L. agilis from Snogeholm in the Zoological Museum, University of Lund.

Description: The gametocytes of K. latus have in most cases a broad, oval shape with rounded ends, but sometimes they are bean-shaped (Figs 12-14). Some of them, probably the older gametocytes, have one end tapered and bent (Figs 12, 15). Their cytoplasm contains neither vacuoles nor granules of pigment, but red spots of volutin placed at one or at both ends of the parasite have often been observed (Fig 13).

There is no capsule surrounding the gametocytes, but sometimes a distinct space is observed, which is probably due to the greater contraction of the cytoplasm of the erythrocyte enclosing the parasite or of the parasite itself. The absence of a capsule causes the parasite to stain deeply.

The macrogametocytes stain deep blue in Giemsa. The length of 475 macrogametocytes from 19 host animals varies between 13.1 and 17.7 μm and the breadth between 5.0 and 10.0 μm (mean 14.9 x 7.2 μm). The nucleus, as a rule, is situated centrally or nearly centrally in the gametocyte, but parasites with the nucleus at one end have also been observed. The nucleus is large, of diffuse structure, composed of a light area with distinctly red chromatin granules. It has no clear boundary and is difficult to measure. 25 nuclei measured 3.1-5.4 μm x 4.6-7.7 μm (mean 4.3 x 6.0 μm). The microgametocytes are usually rarer than the macrogametocytes. They are smaller than the macrogametocytes, and in 377 cases from 17 lizards measured 9.6-15.4 μm x 3.5-7.7 μm (mean 13.0 x 5.9 μm). The cytoplasm of microgametocytes stains faintly blue. The nucleus is situated at the centre of the microgametocyte. It is smaller than that of the macrogametocyte, more compact and of a bright red colour (Fig 15). The size of 20 nuclei varied between 3.1-4.6 μm x 3.8-6.2 μm (mean 3.6 x 4.5 μm).

Trophozoites: The trophozoites are broadly oval. They have pale vacuolated cytoplasm and large reticular nuclei, centrally placed. Twenty trophozoites measured 10.0-13.5 μm x 5.0-8.5 μm (mean 11.9 x 6.4 μm) (Fig 16).

Effects of the parasite on the host cell. Gametocytes cause their host cells to hypertrophy, and to stain paler than uninfected ones. The host cell nucleus is always displaced by the parasite. In most cases it is elongated and compressed and more intensely stained than the nucleus of an uninfected erythrocyte (Figs 12, 14-15). Rarely it is more voluminous and swollen and paler than that of a normal blood cell (Fig 13). It is generally pushed to one of the long sides of the parasite, lying close to its surface, but sometimes it is displaced to one of the ends. Infected erythrocytes with the nucleus divided into two parts may also be noted (Fig 14). Twenty host cell nuclei measured 11.2-16.9 μm x 1.9-3.8 μm (mean 13.1 x 2.8 μm). The size of 20 normal erythrocyte nuclei varied between 5.0-7.7 μm x 3.1-5.0 μm (mean 6.6 x 4.2 μm). The measurements refer to material from Lacerta agilis.

Schizonts: A few schizonts were found in sections of lung and liver, being most numerous in the lung. They were ovoid bodies of one kind, and six schizonts measured 13.1-20.8 μm x 8.5-12.3 μm, containing 8-24 nuclei (Fig 17,18). In a smear of blood from the heart a long and narrow parasite was observed, probably a merozoite. It had a light blue-stained cytoplasm and the nucleus was central and distinctly demarcated. The length of merozoites from the lung was about 9 μm.

General: The infection is usually very light. Seldom more than 2 blood cells out of one thousand were infected. Occasionally two parasites were present in a single cell.

Discussion: As is clear from the description, K. latus differs from K. lacazei and K. lacertae very distinctly as regards the shape, the colour of the cytoplasm and the appearance of the nucleus. Only three species of haemogregarines described from Lacerta resemble K. latus, viz. Haemogregarina nicollei França, 1909, H. nobrei França, 1912 and H. mellisselensis Georgevich, 1940. H. nicollei was described from L. ocellata Daudin, Portugal. According to the detailed original description (França 1909) the cytoplasm of H. nicollei contained no volutin spots. The gametocytes measured 12-15 μm x 4.5-5.5 μm. Thus they seem to be more narrow than those of K. latus. The only kind of schizonts was found in the liver. Like K. latus H. nobrei from L. muralis Laurenti, Portugal had volutin in the cytoplasm, and the same applied to H. mellisselensis from L. serpa Raf. var Galvagnii Werner from islands in the Adriatic. However, H. nobrei had a compact nucleus and both these species differed from K. latus as regards the schizonts which were of two kinds and were found in the liver. As K. latus does not quite agree with any of the species described above, it is regarded as a new species.

4. Karyolysus minor sp. nov.

Host: Lacerta agilis L.
Localities: L. agilis (1 specimen) from Mols, Jutland, 1966; L. agilis (1 specimen) from Svanninge, Funen, 1966, L. agilis (1 specimen) from Degeberga, Scania, 1967.

Type: Slide from L. agilis from Degeberga in the Zoological Museum, University of Lund.

Description: The gametocytes of K. minor are oval, pear-shaped or sometimes bent and with one thicker end. Round forms have been observed. The cytoplasm stains faintly red with Giemsa's stein. The nucleus is diffuse, sometimes band-shaped, sometimes consisting of granules surrounding the parasite. It is red (Figs 19-20). It has not been possible to distinguish macro- and microgametocytes. Seventy-six gametocytes from 4 L. agilis measured 4.6-7.7 μm x 3.1-5.4 μm (mean 6.0 x 3.9 μm).

Trophozoites: Parasites with strap-shaped nuclei are probably young stages, as this kind of nuclei was observed in the first gametocytes that appeared in an infected lizard.

Effects of the parasite on the host cell. The gametocytes live in the cytoplasm of the host cell, which retains normal size and stains normally. The host cell nucleus has its original position and size. Leucocytes are also infected. (Fig 20).

Schizonts: Sections of liver, lung, heart, intestine, spleen and testicle were examined and the schizonts found were typical for K. lacertae. This species was also present in the blood of the examined lizard. No other schizonts were noted. But a mass of round bodies loaded with chromophil granules were found in the lung (Fig 21). Twenty pigmented bodies measured 7.7-11.5 μm x 6.9-10.8 μm. It is interesting to note that França (1909) found pigmented cells of this type in the liver of L. ocellata Daudin infected with Haemogregarina minuta França, a parasite, resembling K. minor in size and effect of the host cell. Mackerras (1961) found that schizonts of H. shattocki Sambon & Seligman from Australian snakes were contained in a mass of cells filled with pigment.

Discussion: K. minor differs very distinctly from K. lacertae, K. lacazei and K. latus as regards size and the effect on the host cell. Haemogregarines resembling K. minor were described from lizards in south Europe.

França (1909) named H. minuta from L. ocellata in the Iberian Peninsula. It measured 7.5-8 μm x 1.5 μm. H. minuta does not change the host cell or its nucleus. França (1912) described H. nana from L. muralis, Portugal. It is more similar to the parasite found in Scandinavia and measured 5.2-6 μm x 3.5 μm. França (1908) finally described H. pallida from Psammodromus algirus L. in Portugal. Nor does this parasite change the host cell. The measurements were 6-7.5 μm x 3 μm, in some cases the breadth was 4 μm. The parasite was oval, pear-shaped or bent with separated ends.

França (1912) mentioned a "H. parva" but it has been impossible to find any description of this parasite, so it seems to be a nomen nudum.

It is doubtful whether these haemogregarines, described by França, are different species. França was inclined to describe as a new species any parasite found in a new host. Whether some of França's parasites is identical with the one described above cannot be determined with certainty: Therefore the Scandinavian species is regarded as a new species.

DEVELOPMENTAL STAGES OF KARYOLYSUS IN MITES

Smears of mites and mites dissected in physiological saline 1-2 hrs after feeding on infected lizards contained many free vermicules from the gastric ceca. Intracellular gametocytes were seldom observed in mites. Thus the liberation of gametocytes took place in the stomach immediately after blood-sucking. However, the encapsulated gametocytes of K. lacertae became free later than those of K. latus and K. lacazei.

The one end of the motile gametocytes of K. latus, especially the microgametocytes, was pointed, the other more rounded. Some motile forms of K. latus were spindle- or cigar-shaped (Figs 22, 25). Macrogametocytes of K. latus produced vermicules 16.9 to 25.4 μm long by 3.8 to 7.7 μm wide (mean 21.8 x 6.0 μm; n = 20 (Fig 22). Free microgametocytes were 12.3 to 23.8 μm by 3.5 to 5.4 μm (mean 17.8 x 4.1 μm; n = 10). (Fig 22). The cytoplasm was blue in Giemsa's stain and sometimes red volutin was visible. The nucleus of the macrogametocytes was diffuse and composed of red chromatin granules. The free microgametocytes have a more compact nucleus (Fig 22).

Free macrogametocytes of K. lacazei measured 14.6-23.1 μm x 2.3-3.8

μm (mean 19.1 × 2.8 μm; n = 20). Microgametocytes were 11.5–16.9 μm × 2.3–3.1 μm (mean 14.2 × 2.6 μm; n = 6) (Fig 23). During the first day after feeding spherical cells were seen. They were apparently contracted and rounded vermicules. Other vermicules, while still elongated, were contracted and thickened at one end. The nucleus of free gametocytes of K. lacazei was situated at the one end or surrounded the light cytoplasm (Fig 23).

The free gametocytes of K. lacertae are similar to those of K. lacazei. The macrogametocytes produced vermicules 11.5 to 17.7 μm long by 2.7 to 4.2 μm wide (mean 15.0 × 3.3 μm; n = 25). Vermicules from microgametocytes were 13.1 to 16.9 μm long by 1.5 to 2.7 μm wide (mean 15.3 × 2.0 μm; n = 15). In the species examined conjugating macro- and microgametocytes were observed within the first day after feeding. Some smaller vermicules, apparently the male gametocytes, were seen adpressed to the surface of the spherical, contracted and rounded female vermicule. Between 25 and 30 hours after feeding unchanged vermicules were present but in greatly reduced numbers. As these first stages of conjugation seldom are observed in the smears, the gametocytes must very soon change shape. Within 48 hours after feeding, most developing forms were shortened and thickened, approaching a spherical shape. Frequently smaller spherical microgametocytes were seen adpressed to the surface of rounded macrogametocytes which changed to single macrogametes. After the nucleus had approached the surface, the macrogamete was mature for fertilization. The microgametocyte produced microgametes by nuclear division (Fig 24). Reichenow (1913, 1921) observed the nucleus of the microgametocyte to divide once, resulting in two microgametes. He described this nuclear division as a single division into two masses of chromatin of the same size (1913). He could not determine the number of chromosomes (1921). My observations are similar to those of Reichenow. Chromosomes were observed only once, obviously in a macrogametocyte (Fig 25). The microgametes were comma-shaped and the length was about 1.9 μm in K. latus and K. lacazei. In one case, two flagellae seemed to be present.

The spherical macrogamete remained motionless, while the microgametes moved actively. They left the microgametocyte, which as a remnant remained adpressed to the macrogamete. The rounded macrogametocytes of K. latus measured from 9.2–13.1 μm in its greatest dimension, the contracted microgametocytes were 5.4–7.7 μm (Fig 24). The same measures were for K. lacazei and K. lacertae 6.2–8.1 μm and 3.5–6.1 μm. Immediately after the penetration of the microgamete the zygote increased in size to 9.2–11.5 μm in its greatest dimension in K. lacazei, to 13.8–14.6 μm in K. latus and to 8.5–11.5 μm in K. lacertae (Fig 26). One to two

days after feeding the largest developing spheres were up to 20 μm in diameter in K. latus and 17 μm in K. lacazei. At this stage the nucleus was spherical with a dense nucleolus (Fig 27). Karyolysus may remain at this stage of development for many days, especially at low temperatures. Sometimes even 8-day-old zygotes were still at this stage. The following development needed higher temperatures (21-30° C). Under such circumstances different stages of development were found 4 to 5 days after feeding. After 7-19 days, the oocysts attained their maximum size, which was for K. latus 25 x 15 μm-52 x 35 μm (mean 36 x 24 μm; n = 15) and for K. lacazei 25 x 15 μm-38 x 38 μm (mean 28 x 24 μm; n = 15).

Reichenow (1921) divided the species of Karyolysus in two groups according to the place in the mite where the conjugated gametocytes enter and grow to oocysts. One group of species develops mainly in the epithelial cells of the stomach, the other type invades the body cavity. He found that K. lacertae, belonged to the first, K. lacazei to the second type. My investigations have verified this. Sections of infected mites revealed developing or mature oocysts of K. lacazei in the podosomal regions of the host coelom (Fig 28). The oocysts of K. latus were usually situated in the epithelial cells of the stomach but were also observed in the coelom. Like those of K. lacazei they were never found in the posterior part of the host, only in the podosoma and anterior opistosoma. Thus the motile sporokinetes had a short way to the eggs of the mite.

Repeated divisions within the oocyst resulted in the formation of 16 nuclei. The divisions of the nuclei took place near the surface of the oocyst. Thus the growing sporokinetes were visible as large protuberances on the surface (Fig 28).

The mature oocysts contained 16 elongated, vermicular, motile bodies. Reichenow (1921) named them sporokinetes, because they give rise to spores in the eggs of the mite. Thus they transmit the infection to the next generation of the mite, where the life-cycle will continue. The sporokinetes were primarily arranged parallel in the oocyst like a bundle (Fig 29). The mature sporokinetes in smears with K. latus measured from 50 x 8 μm to 64 x 8.5 μm (n = 7). Their cytoplasm was pale blue in Giemsa's stain, but the amount of volutin granules was so abundant that the sporokinetes looked red. In the cytoplasm a few, often only two, light blue vacuoles were observed (Fig 30). The nucleus was in most cases central but sporokinetes with the nucleus nearer the one end were observed. The appearance of the nucleus varied. In most cases it was diffuse without a clear boundary, in other cases it was more distinct with small cromatin granules. The nucleolus was visible in sections stained with

Ehrlich's haematoxylin-eosin and it seemed to be situated at the surface of the nucleus (cf. Reichenow 1921). The sporokinetes of K. lacazei and K. lacertae measured 35 x 8 μm to 42 x 8.5 μm; (n = 8) and 36 x 8 μm to 47 x 7 μm; (n = 9) respectively. They had many vacuoles: in the sporokinetes of K. lacertae the vacuoles sometimes formed a net. The volutin was not so abundant as in K. latus and the volutin granules were concentrated around the vacuoles. In mixed infections the sporokinetes of K. lacazei and K. lacertae could not be separated (Fig 31). Seen in phase microscopy the nucleus was spherical with a distinct nucleolus. Spherical as well as short and broad sporokinetes were observed in smears.

The sporokinetes did not mature until the mite again fed on a lizard. Sections and smears made up from 7 to 19 days after the mite had been removed from an infected lizard showed sporokinetes (Tab. 1). Beside mature sporokinetes there were often developing oocysts with protuberances on the surface and zygotes with one nucleus. In one case, cysts with mature sporokinetes were found in sections only 6-7 days after the mite had fed on an infected host and before a new blood-meal. In another case, in sections made up 13-14 days after the mite had been removed from an infected lizard and one day after a new feeding there were no mature sporokinetes. Yet the nuclear division was complete and the sporokinetes were visible as protuberances on the oocyst. Usually smears made up immediately after the second blood-meal contained round or immature sporokinetes, and the first eggs laid one day after feeding were free from sporocysts.

The mature sporokinetes invaded the eggs of mites, where they encysted. Sometimes smear preparations from the eggs made up immediately after oviposition showed sporokinetes becoming shorter, but in other cases encysted sporokinetes were recognised in sections of eggs in the uterus. The encysted sporokinetes, named sporocysts, were oval and in smear preparations they were stained blue in Giemsa's stain (Fig 32). Sporocysts of K. lacazei measured 21.5-26.2 x 11.5-18.5 μm (mean 24.0 x 14.9 μm; n = 12), of K. latus 23.1-32.3 x 15.4-28.5 μm (mean 28.4 x 20.3 μm; n = 12) and of K. lacertae 28.5-33.1 x 16.2-22.3 μm (mean 30.9 x 18.9 μm; n = 12). The nucleus was band-shaped and vacuoles and volutin could be observed in young sporocysts from eggs prepared two days after the second blood-meal. Eggs of mites prepared five days after the females second feeding contained sporocysts of K. latus with 6 visible nuclei and two vacuoles like those of the sporokinetes. Later the vacuoles became numerous and small. Repeated division within the sporocysts resulted in the formation of a number of sporozoites. Smears of nymphs with burst sporocysts showed the number of sporozoites to be 16 in K. lacazei and 32 in K. latus and K. lacertae. The sporozoites of

K. latus measured 19.2 x 7.7 µm to 24.6 x 5.4 µm (mean 21.3 x 5.9 µm; n = 12) and those of K. lacazei and K. lacertae measured 20.0 x 5.4 µm to 24.6 x 4.2 µm (n = 5) and 19.2 x 4,2 µm to 20.0 x 5.4 µm, (n = 7) respectively. Except the nucleus, the sporokinetes contained a vacuole which according to Reichenow (1921) is filled with reserve material (Fig 33). Especially sporozoites of K. lacertae had a large and round vacuole. In mature sporozoites the nucleus was more or less compact. In one case sporozoites from a bursting sporocyst of K. lacertae had nuclei consisting of seven dense, round chromatin bodies.

When the nymphs attacked a lizard for feeding about ten days after the eggs were laid the sporocysts were mature. In smears of two nymphs sporozoites of K. lacertae were found in the sporocysts 5 and 7 days respectively after the maternal mites had fed for a second time. Sporocysts of K. latus contained about 32 nuclei 7 days after the second feeding of the maternal mite.

Usually only a few sporocysts were found in a nymph, but in one case about a hundred sporocysts of K. lacertae were found in the same nymph. Occasionally dead sporocysts were observed in the nymphs.

DEVELOPMENT AND PATHOLOGY OF KARYOLYSUS IN LIZARDS

A number of lizards which had shown no evidence of parasitemia in the laboratory were infected by feeding them experimentally infected nymphs. In a total of 14 L. agilis and 3 L. vivipara the prepatent period was determined. Its duration was as follows: K. latus 32 to 37 days, K. lacazei 30 to 34 days, K. minor 32 to 33 days. K. lacertae was transmitted together with K. lacazei and it was impossible to distinguish trophozoites of these parasites. The prepatent period of mixed infections of K. lacertae and K. lacazei lasted 30 to 31 days (Tab. 2). A few infected nymphs produced infection in the lizard.

Internal organs of the vertebrate hosts were not examined during the prepatent period. Organs from lizards with heavily infected blood showed a great number of schizonts. Thus there was a correlation between the density of gametocytes in the blood and the number of schizonts in internal organs. Lungs, heart, and liver, while containing some schizonts showed few pathological symptoms. One L. agilis in laboratory conditions heavily infected with K. lacertae showed an abnormally enlarged

and fragile spleen. Infected lizards in natural conditions showed no symptoms of disease. Infections of Karyolysus increased to a peak and after a constant level decreased and disappeared.

Acknowledgements - I am grateful to Professor Per Brinck, Head of the Department of Animal Ecology, Lund, for his help and guidance, and to Dr W. Hirschmann, Fürth i. B., Germany who identified the vector, Sauronyssus saurarum.

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Tab. 1. Time of development of sporokinetes of Karyolysus in the mite Sauronyssus saurarum.

Species	Date of first feeding	Date of second feeding	Date of fixation	Days from first feeding to sporokinetes
K. latus	18/12	4/1	5/1	18-19
"	25/3	-	2/4	8-9
"	21/9	-	27/9	6-7
"	26/8	4/9	5/9	10-11
"	26/8	4/9	4/9	9-10
"	27/10	-	4/11	8-9
"	27/10	-	4/11	8-9
"	27/10	-	4/11	8-9
"	27/10	-	4/11	8-9
K. lacazei	26/8	4/9	5/9	10-11
"	26/8	4/9	6/9	11-12
"	15/9	27/9	28/9	13-14
"	24/8	4/9	5/9	12-13
"	15/12	-	28/12	13-14
"	16/11	28/11	29/11	13-14 (oocysts with protuberances)
"	6/8	17/8	18/8	12-13
K. lacertae	18/8	5/9	5/9	18-19
"	15/11	27/11	28/11	13-14
"	15/11	27/11	28/11	13-14

Tab. 2. Experimental transmission of Karyolysus to Lacerta by ingestion of infected nymphs of the mite Sauronyssus saurarum.

Host species	Parasite species	Date of infection	Parasite incidence in circulating blood. Days after exposure										
			30	31	32	33	34	35	36	37	38	39	40
	K. latus												
L. agilis		29/12-59			-		+						
"		31/1 -63	-		+								
"		26/3 -63						+					
"		27/3 -63				-		+					
"		1/4 -63						+		-			
"		27/6 -64						-		+			
L. vivipara		6/5 -65											+5
L. agilis		7/10-67					-	+	+				
	K. minor												
L. agilis		25/10-67				+							
"		28/10-67			+								
	K. lacazei												
L. agilis		25/9 -63	+										
"		7/11-63					+						
L. vivipara		28/4 -63		+									
"		3/5 -65	-		-	+							
L. agilis		3/8 -66		+									
	K. lacazei or K. lacertae												
L. agilis		31/1 -63	+										
"		3/9 -63		+									

- = negative. + = one parasite in count lasting ten or more minutes.
+5 = five parasites in five minutes count.

Fig 1. Gametocytes of Karyolysus lacazei in blood smear of L. agilis
The location of the nucleus in the gametocyte is variable.
n = nucleus of parasite, c = cytoplasm of parasite, d = nucleus of host cell, p = cytoplasm of erythrocyte. Giemsa. 1400 x.

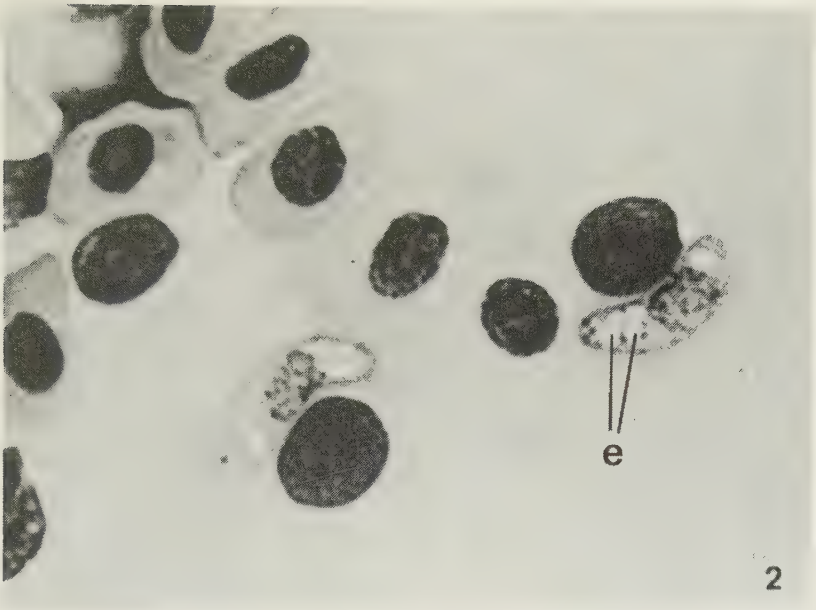

Fig 2. Trophozoites of K. lacazei in blood smear of L. agilis. e = vacuoles. Giemsa. 1400× .

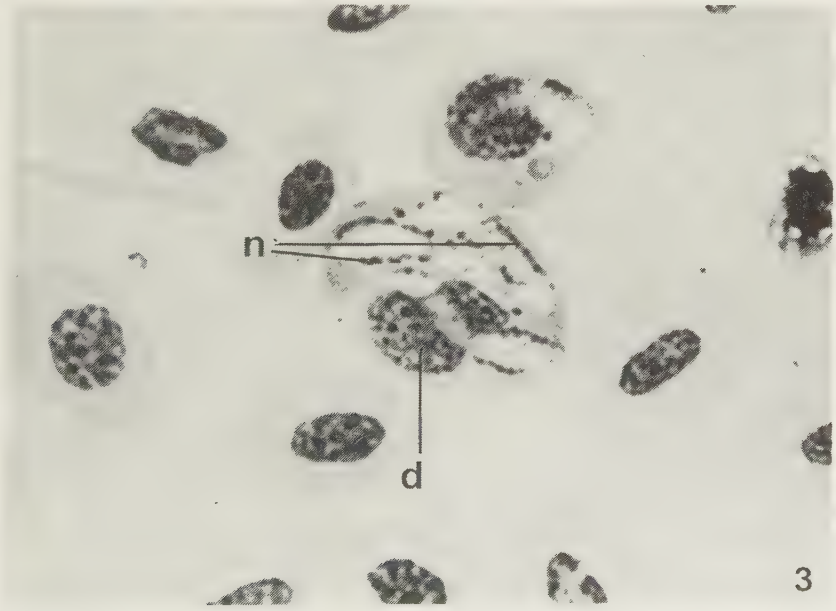

Fig 3. Three gametocytes of K. lacazei in a single cell. Giemsa. 1400 ×.

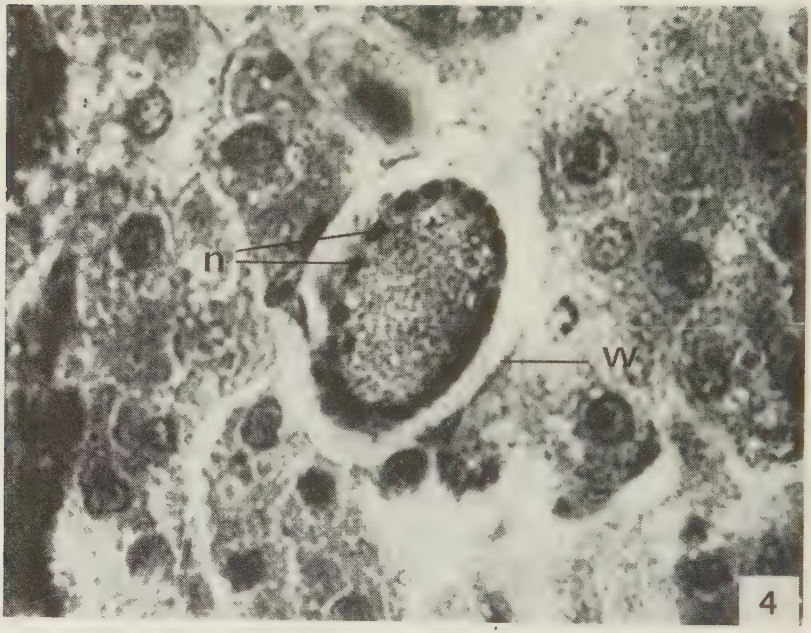

Fig 4. Growing schizont of K. lacazei in liver of L. vivipara. n = nuclei of schizont, w = cyst wall. Fixation Helly. 4μm section. Ehrlich's haematoxylin. 1200 ×.

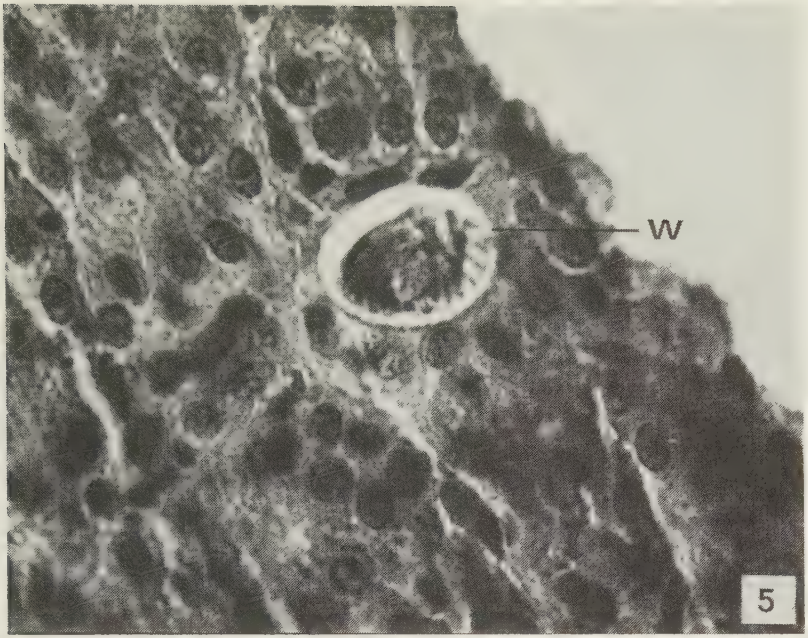

Fig 5. Schizont with merozoites of K. lacazei in liver of L. vivipara. w = cyst wall. Fixation Helly. 5 µm section. Ehrlich's haematoxylin. 1180× .

Fig 6. Gametocyte of K. lacertae in blood smear of L. agilis. n = nucleus of parasite, c = cytoplasm of parasite, a = capsule, d = host cell nucleus, p = cytoplasm of host cell, b = chromophil cap. Giemsa. 2300 ×.

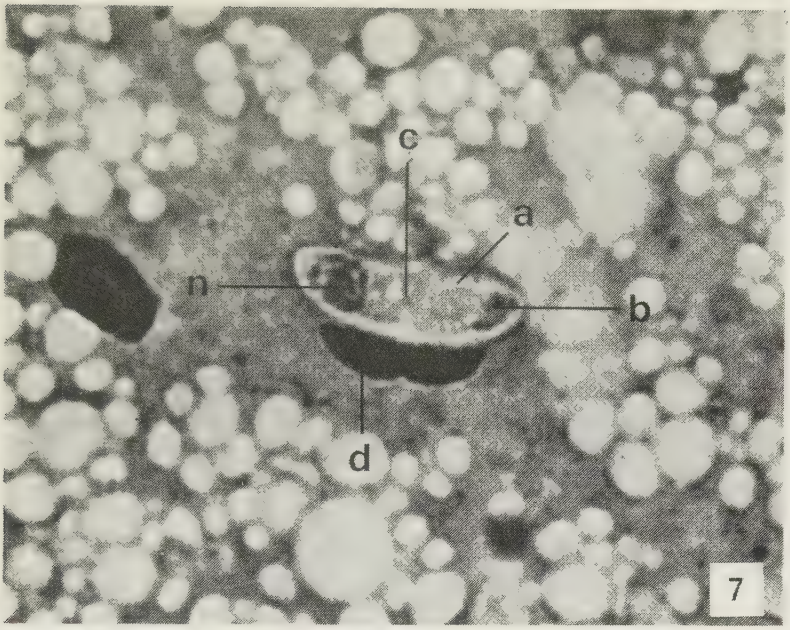

Fig 7. Gametocyte of K. lacertae in liver smear of L. agilis. Giemsa. 2300 ×.

Fig 8. Trophozoites of K. lacertae in blood smear of L. vivipara. e = vacuoles. Giemsa. 2300 ×.

Fig 9. Sexual schizont of K. lacertae in lung of L. agilis. n = nuclei of schizont, w = cyst wall. Fixation Helly. 5 μm section. Ehrlich´s haematoxylin-eosin. 1180× .

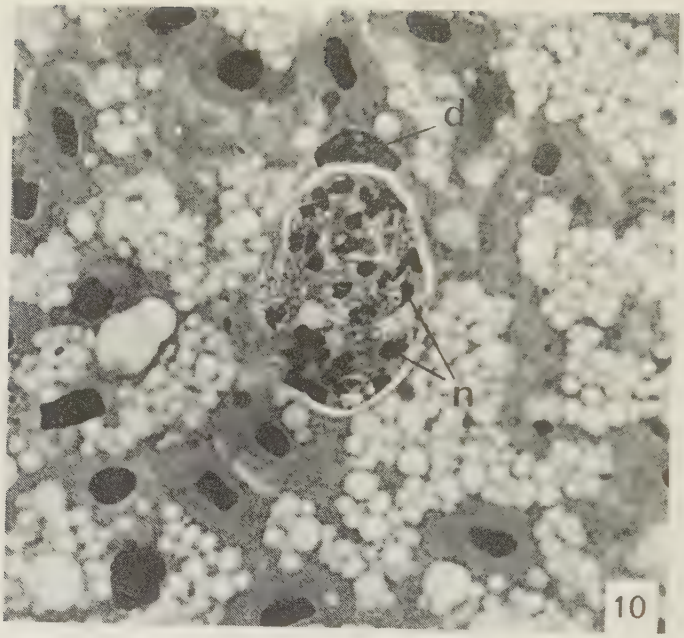

Fig 10. Sexual schizont of K. lacertae in liver smear of L. agilis. n = nuclei of schizont, d = host cell nucleus. Giemsa. 1800× .

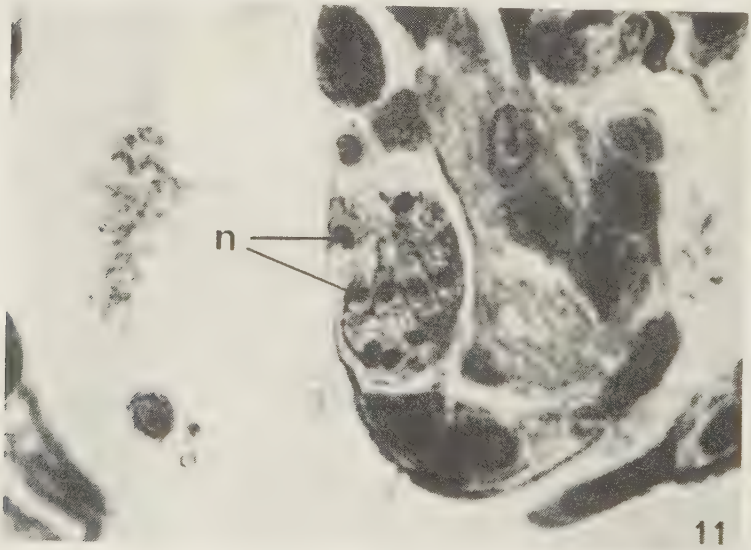

Fig 11. Sexual schizont of K. lacertae in lung of L. vivipara. Fixation Helly. 4 µm section. Ehrlich´s haematoxylin. 1540× .

Fig 12. Macrogametocyte of K. latus in blood smear of L. agilis. One end tapered and bent. The host cell nucleus is compressed. n = nucleus of parasite, c = cytoplasm of parasite, d = nucleus of host cell, p = cytoplasm of erythrocyte. Giemsa. 2240 ×.

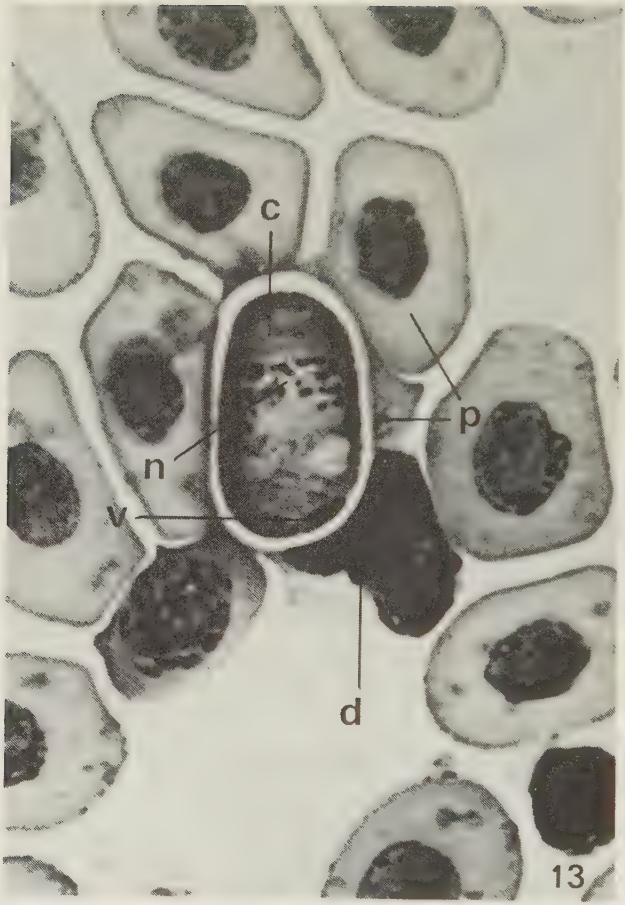

Fig 13. Macrogametocyte of K. latus, nucleus of host cell swollen. v = volutin. Giemsa. 2240× .

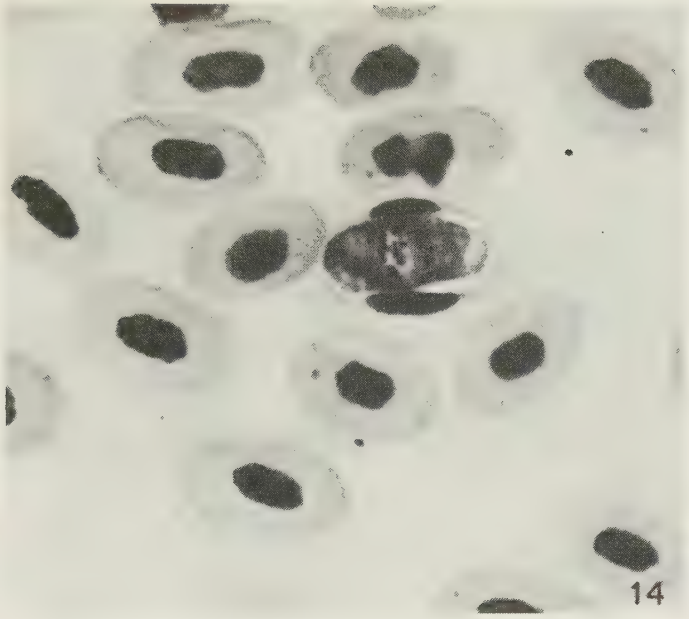

Fig 14. Macrogametocyte, nucleus of host cell divided into two parts. Giemsa. 1400× .

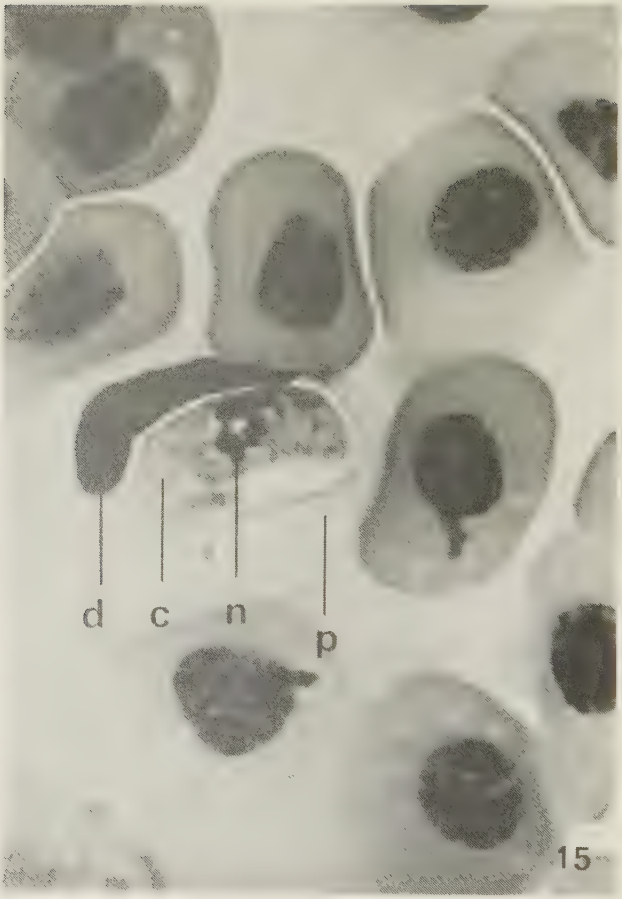

Fig 15. Microgametocyte of K. latus. Giemsa. 2240 ×.

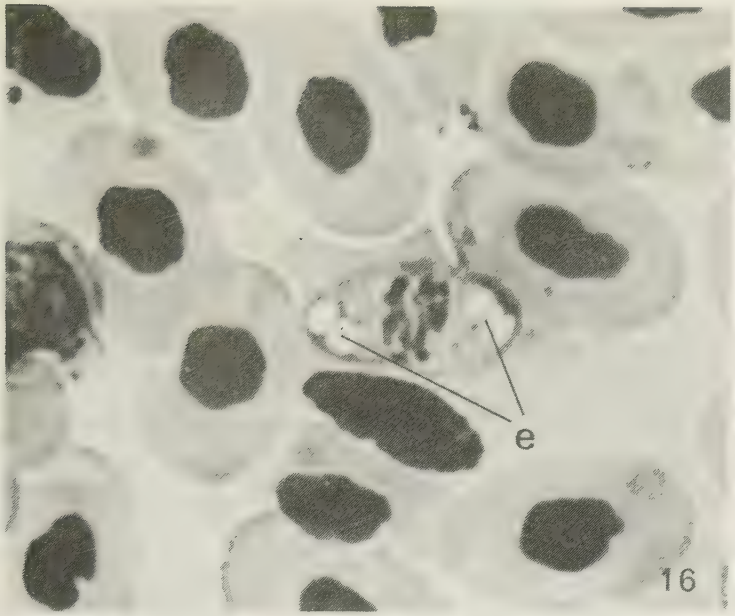

Fig 16. Trophozoite of K. latus. e = vacuoles. Giemsa. 2240 ×.

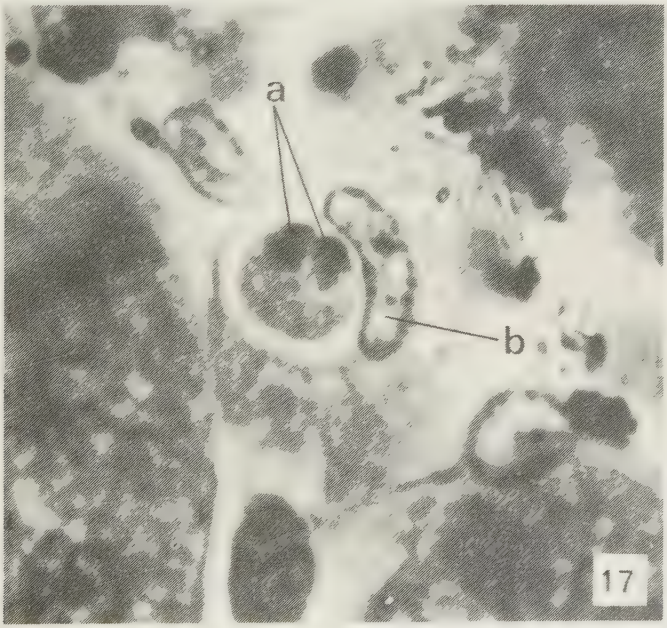

Fig 17. Growing schizont of K. latus in liver of L. agilis. a nuclei of schizont, b nucleus of endothelial cell. Fixation Helly. 8 µm section. Ehrlich's haematoxylin-eosin. 2850 ×.

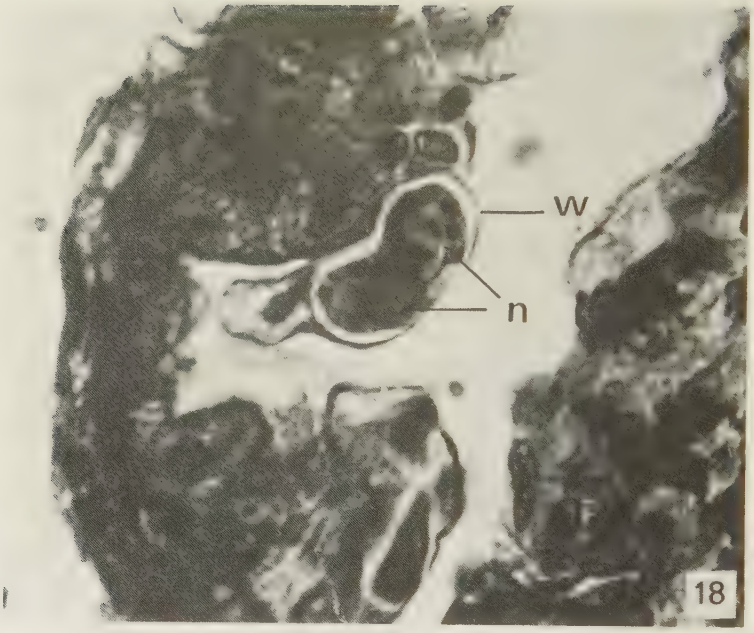

Fig 18. Schizont of K. latus in lung of L. agilis. n = nuclei of schizont, w = cyst wall. Fixation Helly. 6 µm section. Ehrlich's haematoxylin-eosin. 1180 ×.

Fig 19. Gametocyte of K. minor in erythrocyte of L. agilis. n = nucleus of parasite. Giemsa. 1840 ×.

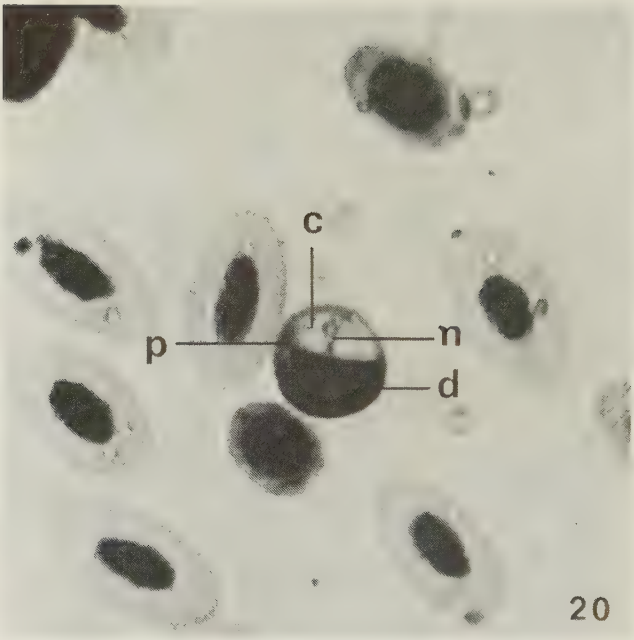

Fig 20. Gametocyte of K. minor in leucocyte of L. agilis. n - nucleus of parasite, d = nucleus of host cell, p = cytoplasm of blood cell. Giemsa. 1840× .

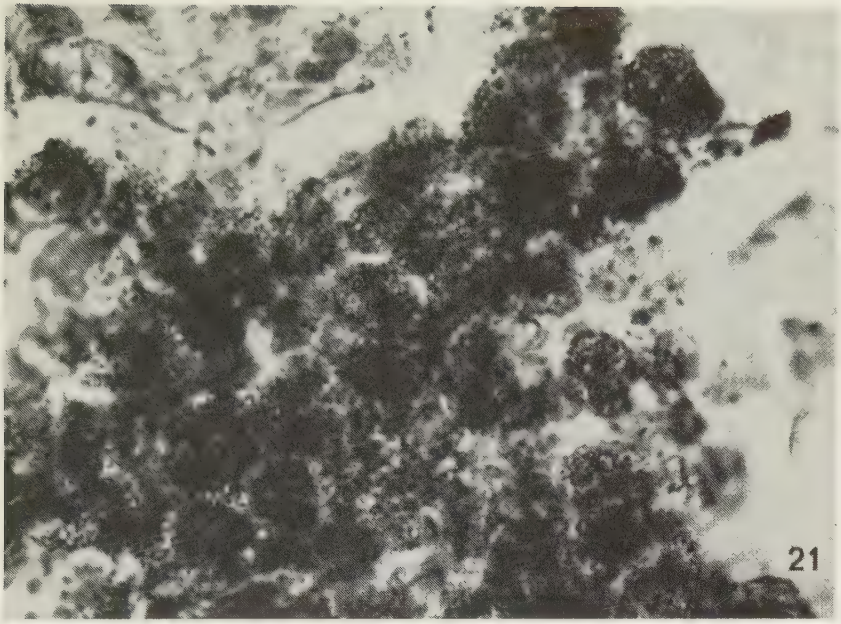

Fig 21. Lung of L. agilis infected with K. minor. Round bodies loaded with chromophil granules. Fixation Helly. 6 µm section. Ehrlich's haematoxylin. 1180 ×.

Fig 22. Free gametocytes of K. latus in smear of Sauronyssus saurarum. n = nucleus of macrogametocyte, m = nucleus of microgametocyte. Giemsa, 2200 ×.

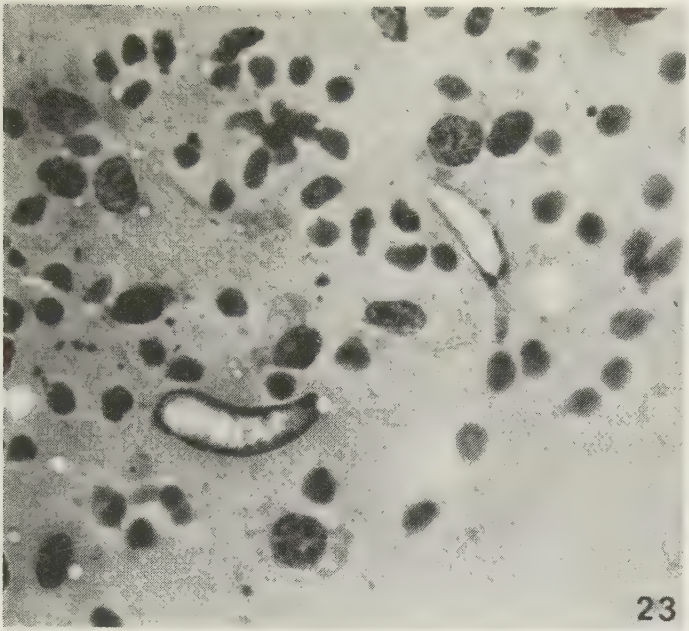

Fig 23. Free gametocytes of K. lacazei in smear of Sauronyssus saurarum. Giemsa. 1180 ×.

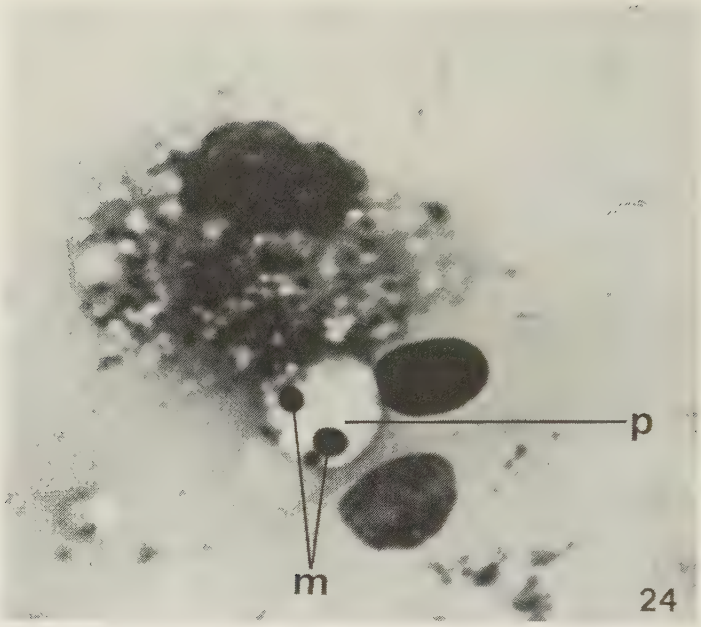

Fig 24. Developing microgametes of K. latus. The microgametocyte nucleus divided into two microgamete nuclei m. p = cytoplasm of microgametocyte. Giemsa. 2200 ×.

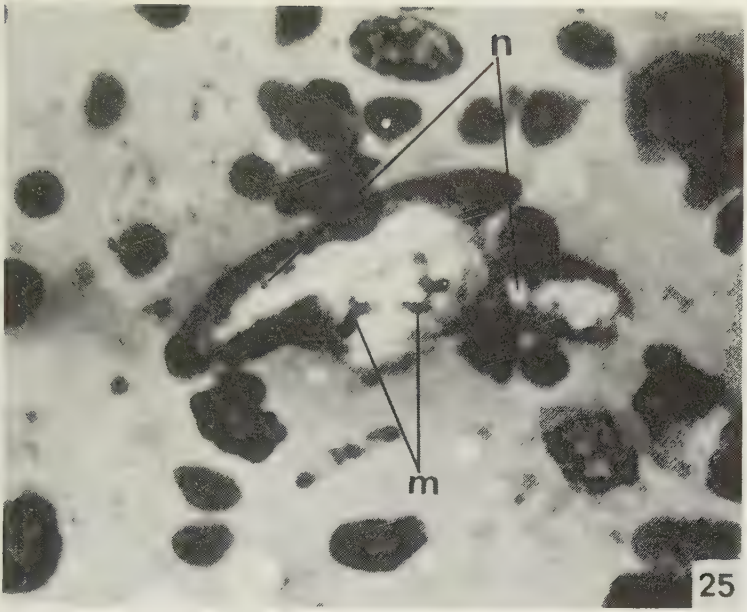

Fig 25. Macrogametocytes of K. latus. n = nuclei, m = chromosoms. Smear of S. saurarum. Giemsa. 2200 × .

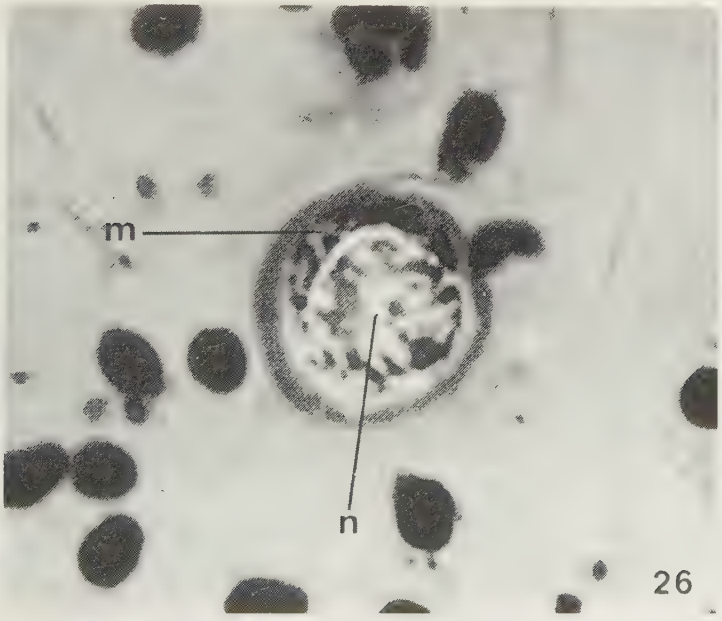

Fig 26. Zygote of K. latus in smear of S. saurarum. The male chromatin m lies like a cap over the female chromatin. Giemsa. 2850 ×.

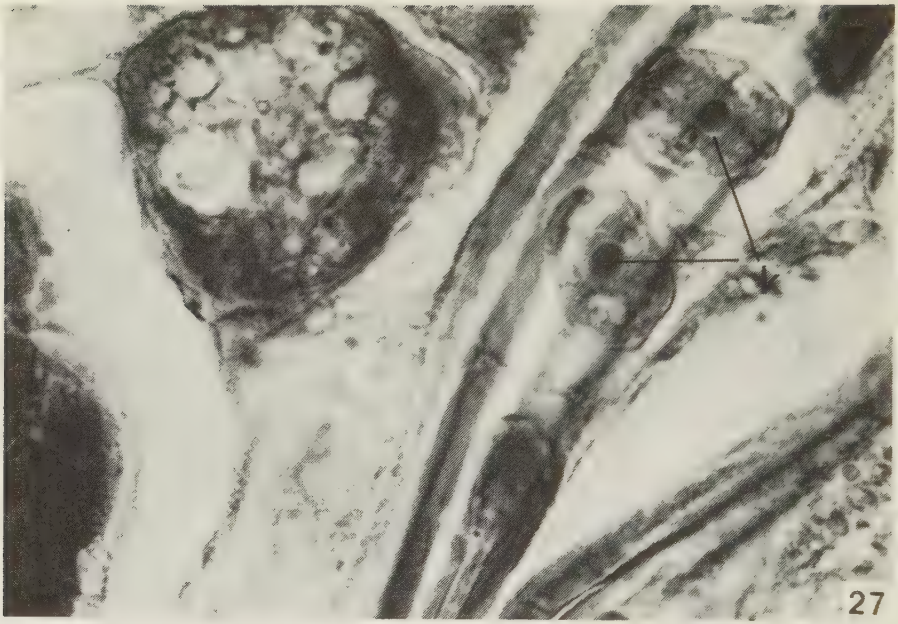

Fig 27. Growing oocysts of K. latus, 2 to 3 days old, in muscles of leg. Fixation Helly. k = nucleolus. 5 µm section. Delafields's hematoxylin. 1180 ×.

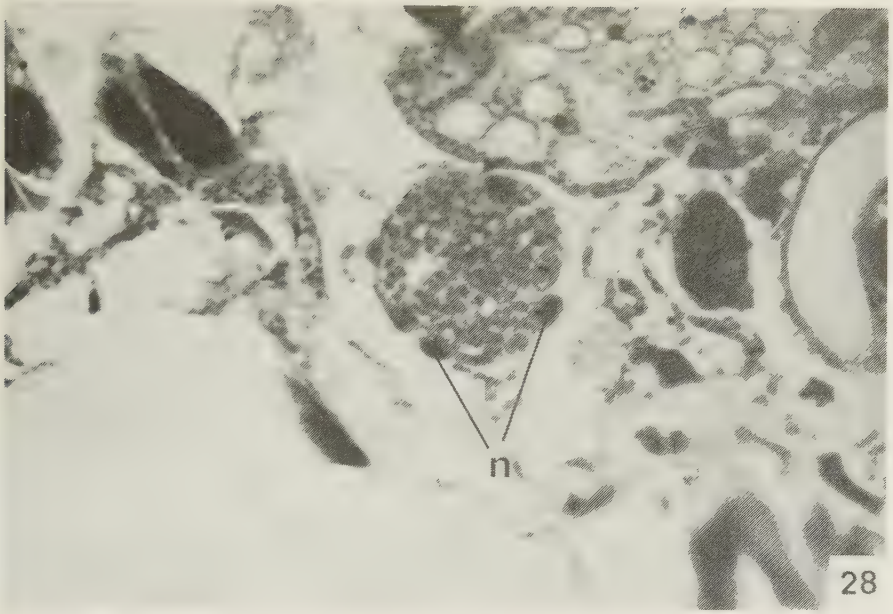

Fig 28. Oocysts of K. lacazei. n – nuclei. Fixation Helly. Embedding Westopal W. 3 µm section. Azan. 1180 ×.

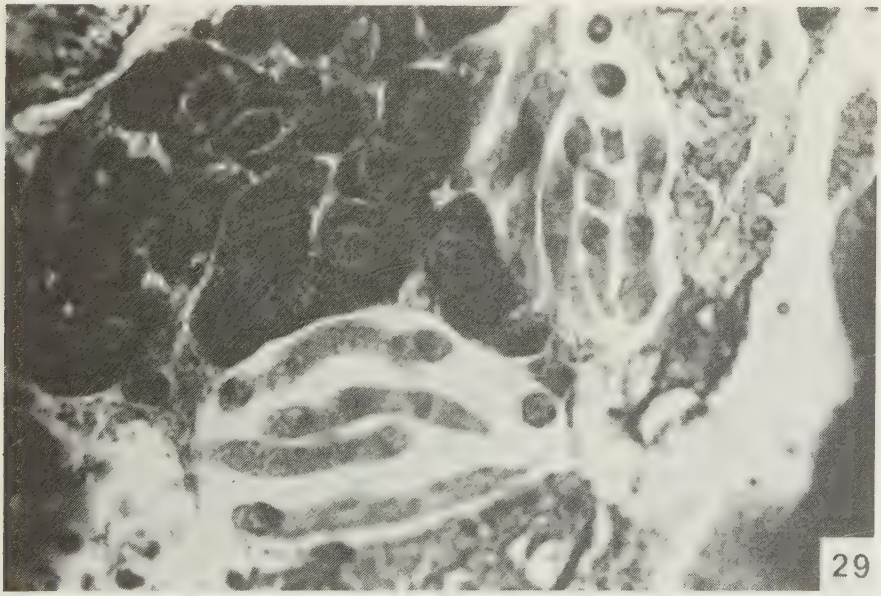

Fig 29. Oocysts of K. latus with mature sporokinetes. Fixation Helly. 5 µm section. Delafield's haematoxylin. 1180 ×.

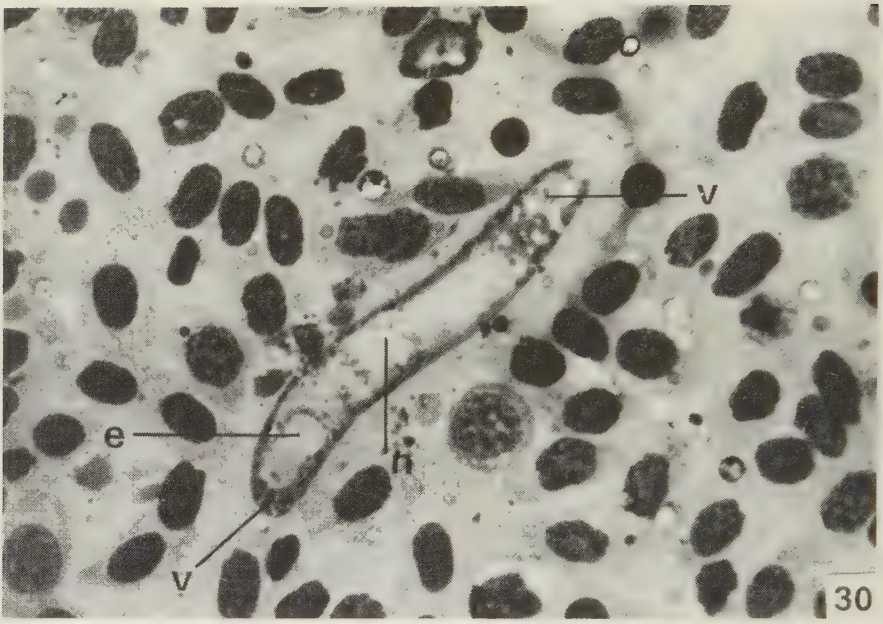

Fig 30. Sporokinete of K. latus in smear of S. saurarum. n = nucleus, e = vacuole, v = volutin. Giemsa. 1180× .

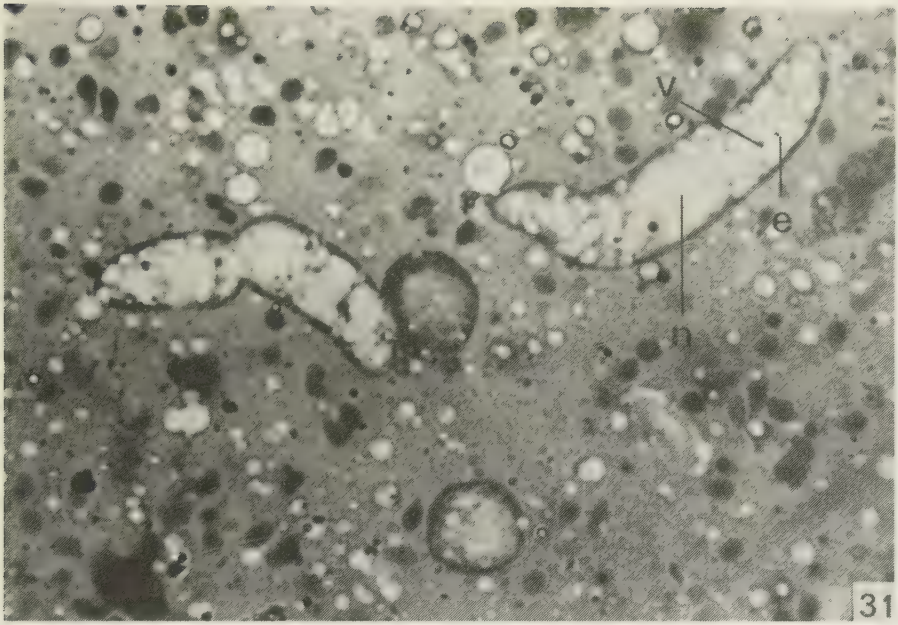

Fig 31. Sporokinetes in mixed infection of K. lacazei and K. lacertae. n = necleus, e = vacuole, v = volutin. Giemsa. 1180 ×.

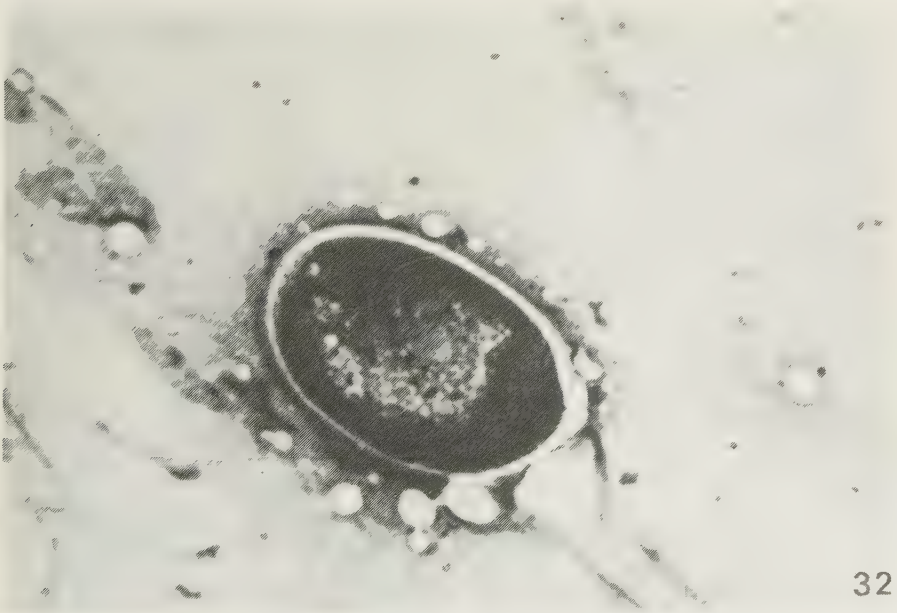

Fig 32. Sporocyst of K. latus in smear of larva of Sauronyssus saurarum. Giemsa. 1440 ×.

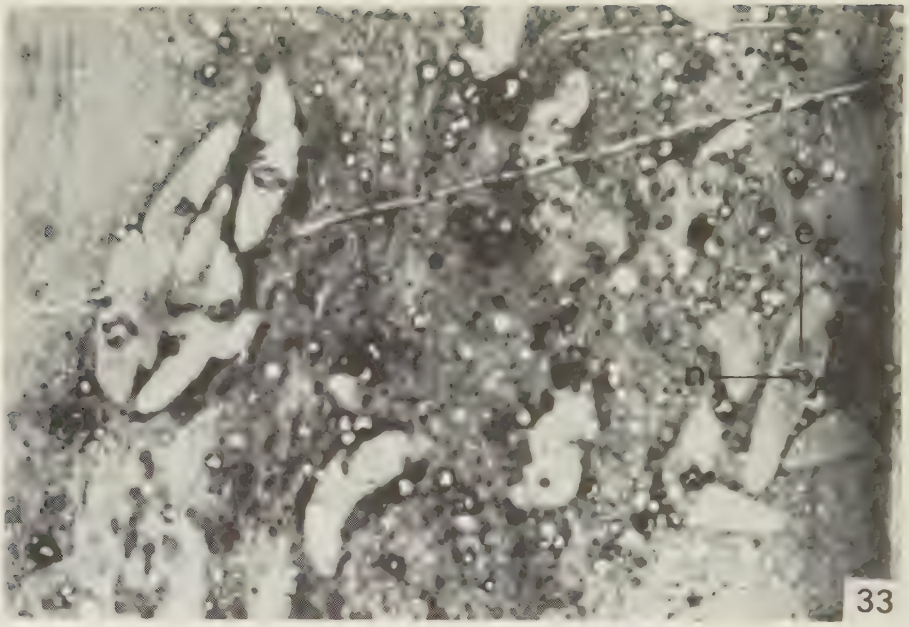

Fig 33. Sporozoites of K. lacazei in smear of larva of S. saurarum. n = nucleus, e = vacuole. Giemsa. 1180 ×.

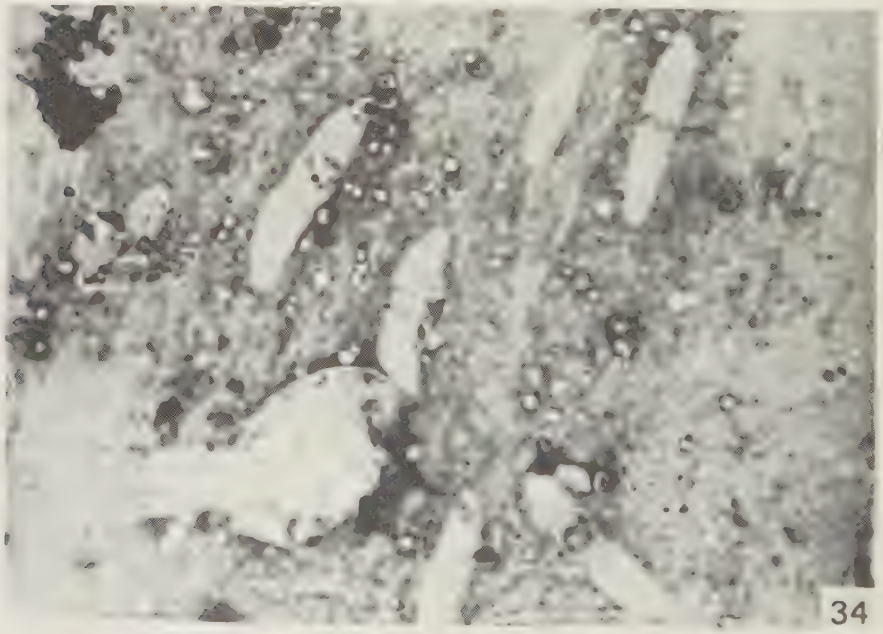

Fig 34. Bursting sporocyst with sporozoites. Giemsa. 1180 ×.

Incidence of Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards

By

Kerstin Svahn

ABSTRACT

Svahn Kerstin 1972. Incidence of Blood Parasites of the Genus Karyolysus (Coccidia, Adeleidae) in Scandinavian Lizards. Four species of Karyolysus were found in populations of lizards from southern Sweden and Denmark. The mean incidence of Karyolysus latus Svahn was 15 % in L. agilis L. and 3 % in L. vivipara Jacquin. The incidence of K. lacazei Labbé was 24 % in L. agilis and 8 % in L. vivipara, and the incidence of K. lacertae Danilewsky in L. agilis was 1 %, and of K. minor Svahn 3 %. K. lacertae and K. minor were never found in L. vivipara.

Variation in gametocyte size between different localities was not statistically significant.

The appearance of initial infections in August and September, the persistence of gametocytes in hibernating lizards and the great number of the transmitting mites in early spring suggest a period of spring transmission.

In laboratory natural infections rose the first two months, remained at a more or less high level for a two-months period and then decreased and disappeared five months later. Reinfections were possible but some resistance was developed.

INTRODUCTION

Species of Karyolysus Labbé, 1894, were reported to parasitize lizards (Lacerta spp.) in southern Europe. Reichenow (1913, 1921) and Svahn (ms) studied the complicated life history of these parasites and demonstrated their arthropod transmission.

Haemoprotozoa were first found in Scandinavian species of Lacerta in blood films from L. agilis L. from Scania, southern Sweden. In 1949-1970 159 L. agilis and 37 L. vivipara Jacquin from 29 localities were investigated. Four species of Karyolysus were found in southern Sweden and Denmark (Svahn, ms). The mite Sauronyssus saurarum Oudemans acted as vector. The present paper describes occurrence and distribution of Karyolysus. Lizards were infected in laboratory and natural and laboratory infections were followed. Seasonal appearance of Karyolysus in Lacerta and the course of the infection were investigated.

MATERIALS AND METHODS

The lizards were collected during the warm season of the year. Experimental animals very soon became adjusted to laboratory conditions and were easily maintained in captivity. At least two-thirds of the specimens were kept in captivity for periods of 1 to 2 years and some specimens for over 4 years.

The lizards were maintained in glass vivaria with a layer of sand in the bottom and placed in water basins in order to prevent mites from transmitting blood parasites between the cages. In winter heat and light were provided by hanging a light bulb over the cage. This gave a temperature gradient ranging from approximately 38° C to room temperature. The light was switched off at night. In summer, lizards were maintained in wire cages outdoors.

The food of the captive lizards consisted mainly of earthworms, but sometimes they were fed mealworms (larva of Tenebrio molitor L.). In the winter vitamins were added.

The intermediate host is a mite, Sauronyssus saurarum Oudemans of the family Gamasidae. It was collected from the lizards as follows. After capture the lizards were placed in a glass vessel standing in water. In the morning mites present crawled on the walls of the vessel and were easily collected in a test-tube with damp filter-paper. Mites were given blood meals by placing them in vessels with lizards.

Blood was drawn from the lizards by clipping the tail-tips. The blood smears after drying were fixed in methanol and stained with Giemsa. Internal organs were fixed in Helly's fixative and 4-8 µm sections were prepared according to the usual paraffin method. Several different stains were used, of which the best proved to be 1) Ehrlich's haematoxylin-eosin, 2) Delafield's haematoxylin, 3) Delafield's haematoxylin-Orange G-Light Green, 4) the Azan-method of Heidenhain, 5) PAS (Periodic acid-Schiff reaction), 6) Gomori's chrome alun haematoxylin (Romeis 1968, Pearse 1960).

A relative estimate of the degree of infection was obtained by five-minute microscope counts of the number of parasites in the blood smears, using 12 x ocular and 42 x objective. Seemingly negative slides were examined for at least 10 minutes. Any gametocyte seen during this time resulted in a rating of +.

STUDY AREA AND HOST MATERIALS

The Sand Lizard, Lacerta agilis, occurs in southern Scandinavia and on warm, sandy slopes facing south and on open sea shores. The Common Lizard, L. vivipara, is widespread in Sweden and Denmark and dwells in many different habitats.

From July 1949 until September 1970 159 L. agilis were collected from 21 localities (1-8, 15, 17-26, 28) and 37 L. vivipara from 13 localities (1, 5, 9-14, 16-18, 26, 27) (vide Tab. 1, 3, Fig 1).

INCIDENCE OF KARYOLYSUS IN SWEDISH AND DANISH POPULATIONS OF LACERTA

The known distribution of Karyolysus in southern Sweden and Denmark is shown in Tables 2, 3 and Fig 1.

Karyolysus in L. agilis: 24 animals from 6 localities were infected with K. latus Svahn, 38 lizards from 6 localities were infected with K. lacazei Labbé, 2 lizards from one locality with K. lacertae Danilewsky and 3 lizards from 3 localities with K. minor Svahn. Double infections were found in 4 animals. Thus, of the specimens examined, 63 (40 %) were infected with Karyolysus. About 20 hatchlings from Snogeholm and Maglehem, examined during their first year were negative and were not included in Tab. 2. Eight localities with 29 lizards investigated were negative.

K. latus, the common species in Scania, was found outside Scania only at Gilleleje, Denmark. The infection rates of Maglehem (92 %) and Degeberga (100 %) populations were higher than in the other sampling areas. The only negative specimen from Maglehem was collected 0.5 km from the infected locality at Maglehem. In Snogeholm only 6 specimens of 51 examined (12 %) were infected. The density of the lizard populations at the sampling time does not explain the high rates of infections at Maglehem and Degeberga, since lizards were numerous also at Snogeholm, while at Gilleleje, where the only animal examined was positive, there were few lizards. Decisive might be the density - and the degree of infection - of the vector, the mite Sauronyssus saurarum Oudemans. Thus, in Maglehem about 250 nymphs of this mite were collected from one lizard in May 1965, while few mites were found in Snogeholm. Instead larval ticks, Ixodes ricinus L., often attached to the lizards from Snogeholm.

The samples from other localities of K. latus are too small for comparison. The infection may be widespread in Scania, though some samples were too small for this to be ascertained.

K. lacazei is the most common species in Denmark. It was not found in Scania, while 2 lizards from Skillingaryd, Småland were positive. At Melsted, Bornholm, all animals examined proved infected. From the Tejn area 11 lizards of 19 examined (58 %) were positive. Five animals (100 %) from Vordingborg and 6 of 8 (75 %) from Anholt were infected. One of 2 lizards collected in Svanninge, Funen was positive. Neither K. latus nor K. lacazei have been found in Jutland.

K. lacertae was found in 2 lizards from the vicinity of Tejn, Bornholm. In both hosts there were multiple infections with K. lacazei and K. lacertae.

K. minor was noted from Degeberga in Scania, where one lizard of 3 examined was infected. The lizard was also positive for K. latus. One lizard in Funen was infected with both K. lacazei and K. minor. In Mols, Jutland, one lizard of 7 examined was positive.

Karyolysus in L. vivipara: Only 4 animals from two localities were positive for Karyolysus. The only infected specimen from Scania was collected at Degeberga, from among L. agilis, which were all infected with K. latus, the infection being very strong. Three of 8 specimens collected at Melsted, Bornholm, were infected with K. lacazei. There were numerous L. vivipara at Melsted, living among L. agilis with a high infection rate.

General

L. vivipara is wide-spread and common in southern Scandinavia occurring as far north as 68° 30′ N, while L. agilis is a local species, restricted to Denmark, southern Sweden and a few localities in central Sweden (Gislén and Kauri 1959). To judge from the present material, the species of Karyolysus in the field seem to be restricted to L. agilis except in habitats where the species of lizards live close together.

In the laboratory there is no difference in susceptibility to Karyolysus between L. agilis and L. vivipara and the vector of the parasite shows no preference of L. agilis.

Nymphs of the vector were obtained from a Karyolysus-negative L. vivipara collected at Genarp in May 1969. Thus, as nymphs do occur on this species, there must be some other reason for the low infection rate of L. vivipara. It may be that the habitats of L. agilis with more continental climatic conditions, are particularly favourable for the development of Karyolysus. Reichenow (1921) found that some stages of development need a high temperature, viz. 25-30° C. My investigations ascertained that temperatures of 21° C are needed (Svahn, ms) and higher temperatures will favour the infection.

VARIATION OF GAMETOCYTE SIZE

Gametocytes of different species of Karyolysus were measured and the measurements were treated statistically. K. lacazei: Length of 200 gametocytes from 11 L. agilis. K. latus: Length and breadth of 475 macrogametocytes from 21 L. agilis and one L. vivipara, length and breadth of 337 microgametocytes from 19 L. agilis and one L. vivipara. In both species measured there was a significant difference both in length and in breadth between gametocytes from different lizards ($P < 0.001$). It was therefore of interest to investigate possible differences between the parasite strains of different lizard populations. Such an investigation was made for K. latus.

The differences between the mean values of the sizes (length and breadth) of the macrogametocytes of K. latus in 17 L. agilis from three localities (Scania: Degeberga, Maglehem, Snogeholm) were subjected to analysis of variance. It was found that the difference as regards the legnth was not significant ($P < 0.2$). Nor was the difference between mean values of macrogametocyte breadth from different localities significant ($P < 0.2$).

There was a significant difference between mean values of microgametocyte length from 16 L. agilis from the three localities ($P < 0.01$).

As regards the size of the K. latus gametocytes of L. agilis in relation to those of L. vivipara, the difference between samples from L. agilis and L. vivipara was not greater than the variation within the Degeberga population in L. agilis.

A comparison of the density of infection and length of macrogametocytes of K. latus in 22 cases (4 localities) gave no correlation. Nor was there any difference in length of gametocytes between mutilple and single infections (K. latus and K. lacazei).

There was no significant correlation between length and breadth of gametocytes of K. latus, the correlation coefficients checked were alternatively positive and negative.

Data on the size correlation between the host cell and the parasite inside it, are not numerous. Beyer (1968) compared dimensions of haemogregarine parasites taken from diploid and triploid species of Lacerta from Armenia. Erythrocytes of triploid lizards were on average one third larger than those of diploid forms. A comparison of sizes of haemogregarines from diploid and triploid hosts revealed no differences within

each form irrespective of dimensions of the cells they lived in. This may be the result of the hereditary stability of gametocytes that are likely to undergo no growing within the host cell.

SEASONAL INCIDENCE OF KARYOLYSUS IN SOUTHERN SCANDINAVIAN LACERTAE

The activities of the lizards and the mite, Sauronyssus saurarum, vary with the season which makes a seasonal variation in the appearance of Karyolysus likely. This variation was analysed for K. latus and K. lacazei which are well represented in the material.

1. Karyolysus latus in L. agilis. The average infection of 73 lizards from six localities was approximately 29 % (21 specimens) (Tab. 4). In May 33 % (2) were infected, in June 17 % (2), in July 33 % (2), in August 25 % (6), and in September 36 % (9). The early samples were small, however, so the lizards collected in May, June and July were pooled. There were 6 (25 %) infected specimens in the material during these months. Three negative animals obtained in August proved to be infected within the prepatent period: 35 days. They have been recorded as negative in Tab. 4. The difference of infection rates between the four months May-August and September is not statistically significant.

The density of infection (Tab. 4) was in the range of + to 10 in 9 of the 21 infected L. agilis examined and from 11 to 55 in the remaining 12/21. During September-May, 10 of 31 lizards showed an 11-55 density range but only 2 of 42 specimens during June-August showed an 11-30 density range.

The infection starts after the lizards have fed on infected nymphs of mites. The natural prepatent period is unknown. In laboratory the time from a first blood meal of female mites on an infected lizard to the appearance of gametocytes in the blood of a second lizard, fed infected nymphs is about 65 days. In nature this cycle is probably longer. Yet it can be expected that the life cycle of Karyolysus is completed in one season.

In laboratory conditions the infection by K. latus increased to a peak and then decreased and finally disappeared (Tabs 6, 7; Fig 2). Apparently this happens in natural conditions too. Disappearing infections were found in 2 lizards from Snogeholm.

The renewal of infections towards the end of the summer is clearly shown for K. latus by the fact that 7 lizards were negative or slightly positive at capture in August and September but gradually built up heavy infections (Tab. 2, Fig 2, A , B, C). One L. agilis (B_2) captured at Maglehem in September 1962 proved on 17.9 1962 to be negative. On 7.10 1962 it was positive for gametocytes of K. latus. In nature this infection would have started in spring 1963 at the earliest, as the lizards begin hibernation in the middle of September. As 2 lizards collected at Maglehem in early May were infected by K. latus, the infection may apparently hibernate in the vertebrate host. Nymphs obtained from a L. agilis collected at Maglehem on 2.5 1965 were eaten by two L. vivipara on 3.5 and on 6.5 1965 respectively. The lizard fed on 3.5 proved still to be negative on 9.6, while the lizard fed on 6.5 was positive for gametocytes of K. latus on 15.6 1965. If nymphs of mites collected at this time of the year were able to infect a lizard, the infection may also hibernate in the vector.

In Scania, lizards begin to be active in the late April or early May. It is probable that the activities of the mites start at the same time of the season. I have observed about 250 mites feeding on a L. agilis in early May.

Hoogstral (1961) on studying Hepatozoon in Egyptian jerboas of the genus Jaculus found that the difference in incidence of infection between summer and winter was statistically insignificant. The infections were denser during winter. Beaudoin, Applegate, Davis, and Mc Lean (1971) and Applegate, Beaudoin, and Seeley (1971) on studying avian malaria found that latent infections of Plasmodium become patent in the spring. This spring relapse of malarial infections coincides with emergence of vectors and may be ecologically important in reestablishing transmission of the parasite if the increase in parasitemia is associated with an increased infectivity of mosquitoes. They also found that birds were significantly more infective to mosquitoes during the spring relapse than during the winter latent period.

Jordan (1970) on studying the occurrence and development of Plasmodium mexicanum Thompson and Huff in the lizard Sceloporus occidentalis Baird and Girard from California found some evidence that the infection was acquired in early spring, since all the three initial infections encountered were from lizards collected in early March.

Apparently the situation of Karyolysus is similar. The fact that all lizards with initial infections of K. latus were found in August and September and that the infections hibernated supports the probability of spring transmission. The gametocytes will be taken up by the vector

when both vertebrate and invertebrate hosts become active in the spring. This conviction is supported by the great number of mites found in early spring and the high infection density during September-May. Then after a long prepatent period during the summer the infection will occur in the autumn or in next spring. Especially last year's hatchlings will show a high infection density as is noted for lizards from Maglehem (Tabs 2, 6). The infection acquired in the spring and recorded in the autumn will disappear during the next summer. This is supported by the fact that infected lizards captured in May and June had almost lost their infections in the following autumn (Fig 2, E, F). Thus disappearing infections from Snogeholm were acquired in the preceding year. Such a seasonal cycle agrees with the fact that lizards collected in spring, summer and autumn had developed infections and that there was no statistical difference of infection rates between the seasons.

2. K. lacazei in L. agilis. The average infection of 30 lizards from five localities was 90 % (27 specimens) (Tab. 5). No animals were collected in May. In June 100 % (5) were infected, in July 70 % (7), in August 100 % (4), and in Sept.-Oct. 100 % (11). Lizards from loc. No 17, Tejn, were excluded, as they did not belong to one population: negative localities were probably included. The density of infection (Tab. 5) was in the range of + to 50 in 4/27 of all infected L. agilis examined, and from 51 to > 300 in the remaining 23/27. During Sept.-Oct., 11 of the 11 lizards showed a 51 to 300 density range but only 12 of 18 specimens during June-Aug. showed a 51 to > 300 density range.

As K. lacazei developed heavier infections, gametocytes persisted for a longer time in the blood and the infection was more easily transmitted in the lizard populations. Thus the majority of lizards had developed infections at capture. The fact that lizards collected in June-Aug. showed a lower infection density suggests a spring transmission. The youngest L. agilis infected was collected at loc. No. 21, Vordingborg in June 1967 and obviously a hatchling from 1966. One trophozoite was noted in a blood smear. The beginning infection had possibly been acquired in early spring, provided that the infection had hibernated in the vector.

THE COURSE OF INFECTION IN LACERTA

The blood of 17 L. agilis and one L. vivipara was examined for a 3 to 25 months period in order to study the course of infection (Tab. 6, Fig 2). Each showed infection at capture or within 35 days after capture (Tab. 2). Of 6 L. agilis (B_1, H_1, H_2, H_3, C_1, K_1) captured in Aug.-Sept. and showing a density within the limits 0-2 at capture, 5 were under observation for 8-9 months. The density rose the first 2 months to 15-20 (H_1, H_2, B_1, K_1) and in the blood of a juvenile lizard (H_3) to 36, remained at that level for a 2-months period, then decreased and disappeared after about 5 months (Fig 2, A, B, C). One animal (C_1) was negative at capture. Two months later it showed a 5 density and 3 months after capture the infection disappeared. No schizogonic stages were found in H_1 when the animal died 17 months after capture.

Seven L. agilis (W37, W25, W45, B_2, B_5, B_6, B_{11}) and one L. vivipara collected in July-Sept. showed a developed infection (Tab. 2). One lizard (W 37) lost its infection a short time after capture and had apparently been infected in the preceding year. Four lizards (W25, W45, B_6, H_4) become negative after 6-9 months. A juvenile animal (B_6) showed a density of 52 at capture and had a 2 density 8 1/2 months later. Another juvenile L. agilis (B_5) showed a density of 52 on the first examination in September. Two months later the density was 105 and 6 months after capture the density was still 22. The only naturally infected L. vivipara was positive 8 1/2 months after capture. Then the infection density had decreased from 60 to 1. When the animal died 14 months after capture the blood was negative. Smears of the organs showed no schizonts. Three (B_1, B_2, B_{11}) were constantly reinfected and showed variation between densities of 1 to 9 for 12 months (Fig 2, D). The organs of one of these lizards (B_{11}) were examined in smears 13 months after capture but no schizonts were found. Blood smears showed a gametocyte density of 2.

Four lizards (B_7, B_9, B_{10}, E_1) were collected in May-June. They showed a decreasing infection which desappeared or was almost lost in the autumn. They were all reinfected (Fig 2, E, F). E_1 became negative 4 months after capture but was reinfected and showed a very weak infection 9 months after capture. B_{10} was reinfected and the density rose from 1 to 5 in the autumn, 4 months after capture. Next summer the infection density was 12. When the lizard died 15 months after capture sections of the organs were examined. Schizonts were found in the lung.

In one L. agilis (H_2) captured in August the density of K. minor varied between 0, 1 and 2 in 4 months.

Three L. agilis (Fn_1, E_1, W45) were examined for gametocytes of K. lacazei for a period of 17 to 25 months (Tab. 7). An adult female (Fn_1) was positive for K. lacazei at capture, 7 months later it was negative, later it was reinfected and when the animal died 25 months after capture it showed a 150 density. Another adult female (E_1) positive for K. latus was infected with K. lacazei and during 15 months the density rose from 2 to 230. A dense infection of K. lacertae could be noted. A great number of schizonts in all stages of development were found when the animal died 17 months after capture. A female L. agilis (W45) positive for K. latus at capture lost this infection and showed trophozoites of K. lacazei in the blood and schizonts in internal organs 2 years after capture. A L. vivipara (A_{10}) positive for K. lacazei showed correlation between the high density of gametocytes in the blood and the great number of schizonts in internal organs. In another L. vivipara (A_8) observed during 3 months the density rose from 0 to 290.

Discussion

During the course of the investigation 20 L. aigilis and 2 L. vivipara naturally or artificially infected with Karyolysus were observed for gametocytes for a period of 2 1/2 to 25 months. It is apparent from the natural infections of K. latus that the number of gametocytes increased to a peak 1 to 2 months after the first positive observation and then remained at a constant level for 2 months. After this maximum level the infection decreased and disappeared 5 to 6 months later. Very heavily infected animals could be positive more than 8 months.

Lizards developed some resistance when they were subjected to repeated infections but those with low grade parasitemias were not resistant to reinfections. Thus the heaviest natural infections of K. latus were developed in juvenile hosts (H_3, B_2, B_5, B_6). By many reinfections the density could be held on a low level for 13 to 22 months (B_1, B_2, B_{10}, B_{11}). Thus, in nature lizards can probably acquire a new infection, although they are already positive. Such a reinfection every spring will secure the infection in the lizard population.

After the parasites had disappeared from the blood, no schizonts were found in internal organs, and the infection did not reappear in the blood, unless the lizard was reinfected. Hosts already infected by a species

of Karyolysus were not resistant to other species. Thus mixed infections were found in nature, and in laboratory conditions all species could occur together. For a short time one L. agilis (H_1) had four species of gametocytes in the blood. The presence of K. latus did not prevent infection by K. lacazei and K. lacertae (E_1), and K. minor was mixed with K. latus (H_1, H_2) and with K. lacazei and K. lacertae (Fn_1, H_1). However, under natural conditions mixed infections were rare (Fn_1, H_2 and two L. agilis from Bornholm) and no more than two species were found in the same host specimen.

As infections of K. lacazei and K. lacertae were heavier than those of K. latus and K. minor, the hosts infected by the former were more easily reinfected when caged with mites. In arthropod-free conditions the infections disappeared.

Organs from heavily infected lizards showed a great number of schizonts. Thus there was a correlation between the density of gametocytes in the blood and the number of schizonts in internal organs. Possibly there was a connection between high density of parasites and sudden death of some animals (E_1, A_{10}).

Even at heavy infections of Karyolysus the lizards showed no symptoms of disease. In laboratory conditions, however, leucocytosis and anemia were clear pathological effects of these parasites.

Acknowledgements - I am greatly indebted to Professor Per Brinck, Head of the Department of Animal Ecology Lund, for his help and guidance. Professor Karl Georg Wingstrand, Copenhagen, Professor Anders Enemar, Gothenburg, and Dr Håkan Hallander, Veberöd, kindly helped in the collecting of material, and I wish to express my deep gratitude to them for their help and interest in my work. I am also grateful to Dr W. Hirschmann, Fürth i. B., Germany, who identified the vector, Sauronyssus saurarum.

The work has been supported by grants from the Faculty of Mathematics and Natural Sciences of the University of Lund, the Royal Physiographic Society, Lund, and The Nordic Council for Terrestrial Ecology.

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Table 1. Collecting locality in Denmark and southern Sweden

Locality	Locality No	Description of site
Sweden		
Scania		
North shore of Lake Snogeholmssjön	1	Sandy slope towards the lake. Vegetation: pine scrub and grass.
Hallamölla	2	Sandy slope towards stream. Vegetation: pines and grass.
Maglehem	3	Sandy slope towards stream. Vegetation: pines and grass.
Brösarp, Fisketorp	4	Sandy slope at a pond. Vegetation: pines and grass.
Degeberga	5	Sandy slope towards stream. Vegetation: pines and grass.
Ignaberga, limestone quarry	5a	Sandy slope at a pond. Vegetation: grass.
Kåseberga	6	Sandy, steep slope towards the Baltic Sea. Vegetation: grass.
Isle of Ven, old church	7	Sandy, steep slope towards the sea. Vegetation: grass.
Glumslöv, Ålabodarna	8	Slopes at a pond and towards the sea. Vegetation: grass.
Genarp	9	Stone dike on pasture.
Södra Sandby	10	Unknown.
Falsterbo	11	Garden with ling.
Häckeberga	12	Unknown.
Skäralid	13	Unknown.

Locality	Locality No	Description of site
Öland		
Möckelmossen	14	Exposed limestone area. Vegetation: low scrub, grass.
Småland		
Skillingaryd	15	Slope towards river. Vegetation: pines and grass.
Edshult	16	Headland and forest.
Denmark		
Bornholm		
Tejn	17	Gardens, headlands, heath. Vegetation: grass, ling.
Gudhjem, Melsted	18	Shore with downs.
Zealand		
Gilleleje	19	Shore with grass.
Stevns klint	20	Precipice towards the sea. Vegetation: grass.
West of Vordingborg	21	Shore. Vegetation: pines and grass.
Mön		
Jydelejet	22	Sandy slope. Vegetation: grass.
Hårbölle harbour	23	Shore. Vegetation: grass.
Samsö		
Vesterlöcken	24	Sandy steep slope towards the sea. Vegetation: pines and grass.
Funen		
Svanninge	25	Sandy slope. Vegetation: grass.

Locality	Locality No	Description of site
Jutland		
Mols bjerge, Knebel	26	Sandy slopes. Vegetation: pines and grass.
Hegedal west of Fjällerup	27	Shore with grass, straw-rick in a farm
Anholt		
West shore of the island		Shore with crowberry, Empetrum nigrum L., sandy slope with grass, pine forest.

Table 2. Species of Karyolysus in Lacerta agilis. Number of parasites counted in 5 min. T = K. latus, Z = K. lacazei, C = K. lacertae, M = K. minor.

Locality No	Number of negative host animals	Infected specimens							
		Date of capture	Date of blood test		Sex and age of lizards examined	Number of specimens of Karyolysus species			
						T	Z	C	M
1	45	10/7 1949	10/7	411	♂ ad.	23	-	-	-
		13/8 1957	13/8	W3	♀ ad.	8	-	-	-
		12/8 1958	13/8	W37	♀ ad.	3	-	-	-
		19/7 1959	20/7	W25	♀ ad.	8	-	-	-
		15/8 1960	19/8	W45	♀ ad.	5	-	-	-
		10/9 1962	11/9	W50	♀ ad.	11	-	-	-
2	1	20/8 1968	21/8	K_1	♂ juv.	2	-	-	-
3	1	17/9 1962	17/9	B_1	♀ juv.	-	-	-	-
			7/10		"	20	-	-	-
		17/9 1962	10/10	B_2	♂ juv.	50	-	-	-
		13/9 1964	29/9	B_5	juv.	52	-	-	-
		"	"	B_6	♀ juv.	52	-	-	-
		"	"		♀ ad.	14	-	-	-
		"	"		♀ ad.	+	-	-	-
		2/5 1965	3/5	B_7	♂ ad.	17	-	-	-
		3/5 1966	4/5	B_9	♂ ad.	11	-	-	-
		1/6 1967	2/6	B_{10}	♀ ad.	10	-	-	-
		14/9 1967	15/9	B_{11}	♂ ad.	19	-	-	-
		7/9 1969	8/9		♀ ad.	16	-	-	-
4	3	aug. 1963	aug.	C_1	♀ ad.	-	-	-	-
			14/10		"	3	-	-	-
		23/8 1969	24/8	C_2	♀ juv.	-	-	-	-
			1/10		"	9	-	-	-
5		21/8 1967	22/8	H_1	♂ ad.	+	-	-	-
		"	"	H_2	♀ ad.	+	-	-	-
		"	19/9		"	11	-	-	+
		"	22/8	H_3	♂ juv.	-	-	-	-
			19/9		"	5	-	-	-

Locality No.	Number of negative host animals	Infected specimens						
		Date of capture	Date of blood test	Six and age of lizards examined	Number of specimens of Karyolysus species			
					T	Z	C	M
5a	2							
6	3							
7	5							
8	6							
15		1960	5/8	♀ ad.	–	100	–	–
		"	"	♀ ad.	–	105	–	–
17	8	24/8 1960	9/9	♂ ad.	–	35	–	–
		"	"	♂ juv.	–	28	–	–
		"	"	♀ ad.	–	83	–	–
		"	"	♀ juv.	–	76	–	–
		7/9 1961	8/9	♀ ad.	–	85	–	–
		juni 1962	20/7	♂ ad.	–	60	120	–
		"	"	♂ ad.	–	+	5	–
		"	"	♂ juv.	–	259	–	–
		"	"	♀ ad.	–	290	–	–
		"	"	♀ ad.	–	83	–	–
		"	"	♀ ad.	–	48	–	–
18		17-21/8-65	27/8	♀ ad.	–	105	–	–
		"	"	♀ juv.	–	112	–	–
		"	20/10	♀ juv.	–	130	–	–
		"	"	♂ ad.	–	140	–	–
		"	"	♂ ad.	–	160	–	–
		"	"	♀ old	–	151	–	–
		"	"	♀ ad.	–	181	–	–
		"	"	♀ ad.	–	64	–	–
		"	"	♀ ad.	–	205	–	–
		"	"	♀ ad.	–	67	–	–
		"	"	♀ ad.	–	140	–	–
		"	"	♀ ad.	–	232	–	–
		"	"	♀ ad.	–	69	–	–

Locality No.	Number of negative host animals	Infected specimens							
		Date of capture	Date of blood test		Sex and age of lizards examined	Number of specimens of Karyolysus species			
						T	Z	C	M
19		12/6 1967	26/6	E_1	♀ ad.	11	-	-	-
20	3								
21		15/6 1967	26/6		♀ ad.	-	290	-	-
		"	"		♀ ad.	-	181	-	-
		"	"		♀ ad.	-	221	-	-
		"	"		♂ ad.	-	145	-	-
		"	"		juv.	-	+	-	-
22	4								
23	2								
24	4								
25	1	1/7 1966	13/7	Fn_1	♀ ad.	-	9	-	1
26	6	29/6 1966	18/7		♀ ad.	-	-	-	1
28	2	13/7 1969	21/7		♀ ad.	-	135	-	-
		"	"		♀ ad.	-	140	-	-
		"	"		♀ ad.	-	430	-	-
		"	"		♀ ad.	-	49	-	-
		"	"		♂ ad.	-	55	-	-
		"	"		♂ ad.	-	2	-	-

Table 3. Species of Karyolysus in Lacerta vivipara. Number of parasites counted in 5 min. T = K. latus, Z = K. lacazei, C = K. lacertae, M = K. minor.

Locality No.	Number of negative host animals	Infected specimens							
		Date of capture	Date of blood test		Sex and age of lizards examined	Number of specimens of Karyolysus species			
						T	Z	C	M
1	5								
5		10/9 1968	11/9	H_4	♂ ad.	60	-	-	-
9	1								
10	2								
11	1								
12	2								
13	1								
14	1								
16	6								
17	5								
18	5	5/9 1964	8/9		ad.	-	40	-	-
		18/8 1965	27/8		ad.	-	58	-	-
		"	"		ad.	-	115	-	-
26	2								
27	2								

Table 4. Seasonal incidence and density (5 min. counts) of Karyolysus latus infection in Lacerta agilis from loc. 1 Snogeholm, 2 Hallamölla, 3 Maglehem, 4 Brösarp, 5 Degeberga, 19 Gilleleje.

Density of infection (5 min. counts)	May	June	July	Aug.	Sept.	Total
Negative	4	10	4	18	16	52 (71%)
+ - 10	-	1	1	6	1	9 (12%)
11-20	2	1	-	-	5	8 (11%)
21-30	-	-	1	-	-	1 (1%)
50-55	-	-	-	-	3	3 (4%)
Total	6	12	6	24	25	73
% total infected		6 (25%)		6 (25%)	9 (36%)	21 (29%)

Table 5. Seasonal incidence and density (5 min. counts) of Karyolysus lacazei infection in Lacerta agilis from loc. No 15 Skillingaryd, 18 Bornholm, 21 Vordingborg, 25 Funen, 28 Anholt.

Density of infection (5 min. counts)	May	June	July	Aug.	Sept.-Oct.	Total
Negative	-	-	3	-	-	3 (10%)
+ - 25	-	1	2	-	-	3 (10%)
26-50	-	-	1	-	-	1 (3%)
51-100	-	-	1	1	3	5 (17%)
101-150	-	1	2	3	3	9 (30%)
151-200	-	1	-	-	3	4 (13%)
201-300	-	2	-	-	2	4 (13%)
> 300	-	-	1	-	-	1 (3%)
Total	-	5	10	4	11	30
% total infected		5 (100%)	7 (70%)	4 (100%)	11 (100%)	27 (90%)

Table 6. Density of K. latus gametocytes in peripheral blood of 17 L. agilis and one L. vivipara. Natural infection.

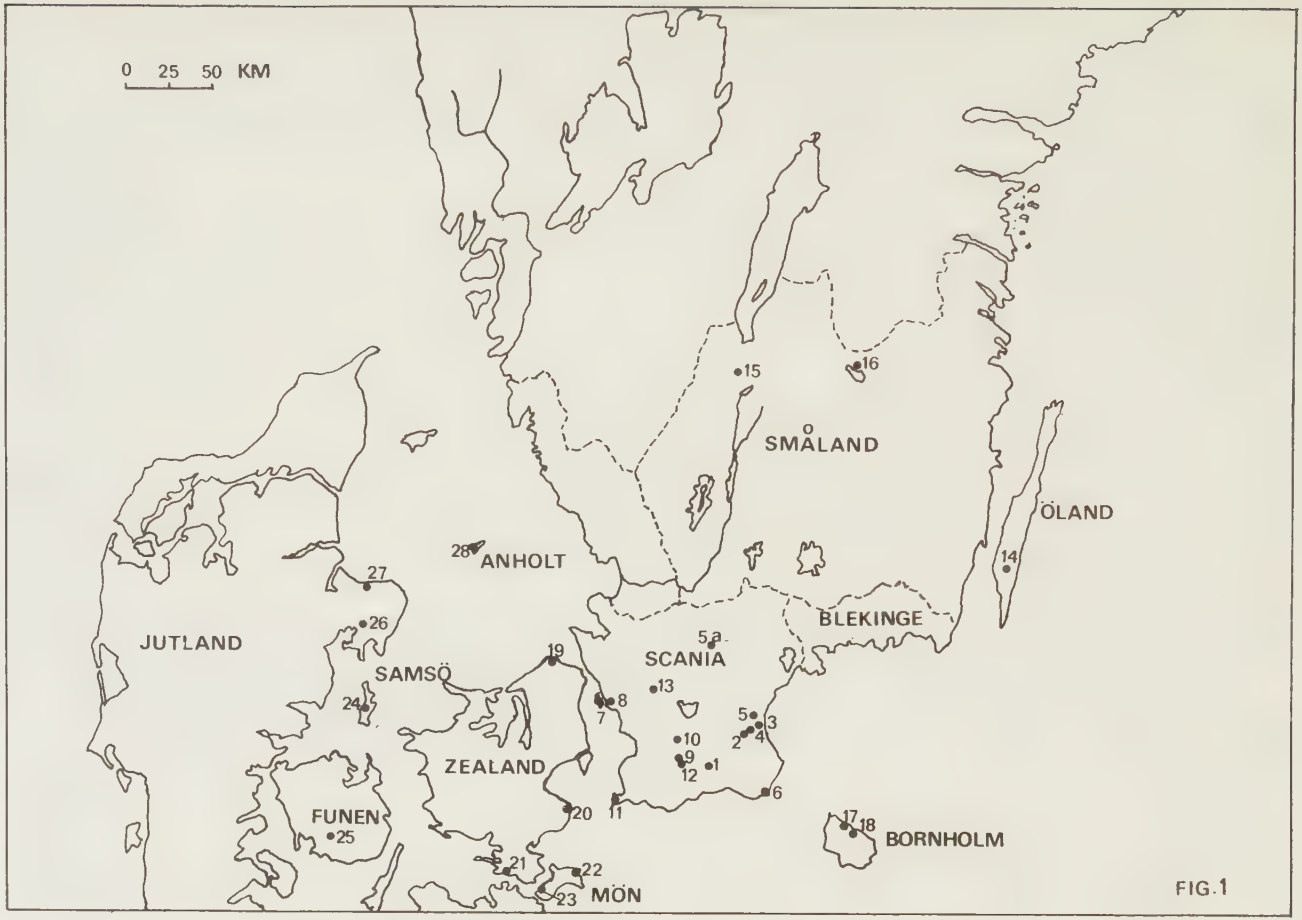
Host number	Density range*	Remarks	Period of observation
H_1	0-17-1	Internal organs after 17 months	August-April 8 months
H_2	1-20-2	Organs not examined	August-April 8 months
H_3	1-36-0	Organs not examined	August-April 8 months
B_1	0-20-0	Organs not examined	September 1962- August 1964, 22 1/2 months
B_2	50-0	Organs not examined	October 1962-April 1964, 18 months
B_5	52-105-22	Organs not examined	September-April 6 months
B_6	52-2	Organs not examined	September-June 8 1/2 months
B_7	17-1-9-1	Organs not examined	May-January, 9 months
B_9	11-1	Organs not examined	May-February, 10 months
B_{10}	10-1-5-12	Organs positive	June 1967-September 1968, 15 1/2 months
B_{11}	19-4-9-2	Organs negative after 13 months	September 1967-October 1968, 13 months
E_1	11-0-1	Organs negative after 17 months	June-March, 8 1/2 months
K_1	2-20-0	Organs not examined	August-May, 7 months
W25	8-1	Organs not examined	July-February, 7 months
W45	5-0	Organs negative after 24 months	August-February, 6 months
W37	3-0	Organs not examined	August-March, 7 months
C_1	0-5-0	Organs not examined	August-November, 3 months
L. vivipara, H_4	60-1	Organs examined in smears. Neg.	September-May 8 1/2 months

* Initial, maximum and last stages density

Table 7. Density of K. lacazei gametocytes in peripheral blood of 3 L. agilis and 2 L. vivipara.

Host number	Density range*	Remarks	Period of observation
Fn_1	9-0-150	Organs positive	July 1966-August 1968, 25 months
E_1	0-350	Organs positive	June 1967-November 1969, 17 months
W45	Trophozoites	Organs positive	July 1960-August 1962, 25 months
L. vivipara, A_{10}	Positive	Organs positive	July 1962-October 1962, 3 months
L. vivipara, A_8	0-290	Organs not examined	3 months

* Initial, minimum and last stages density


0 25 50 KM
SMÅLAND
ÖLAND
ANHOLT
JUTLAND
SAMSÖ
SCANIA
BLEKINGE
ZEALAND
FUNEN
BORNHOLM
MÖN
1
2
3
4
5
5a
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7
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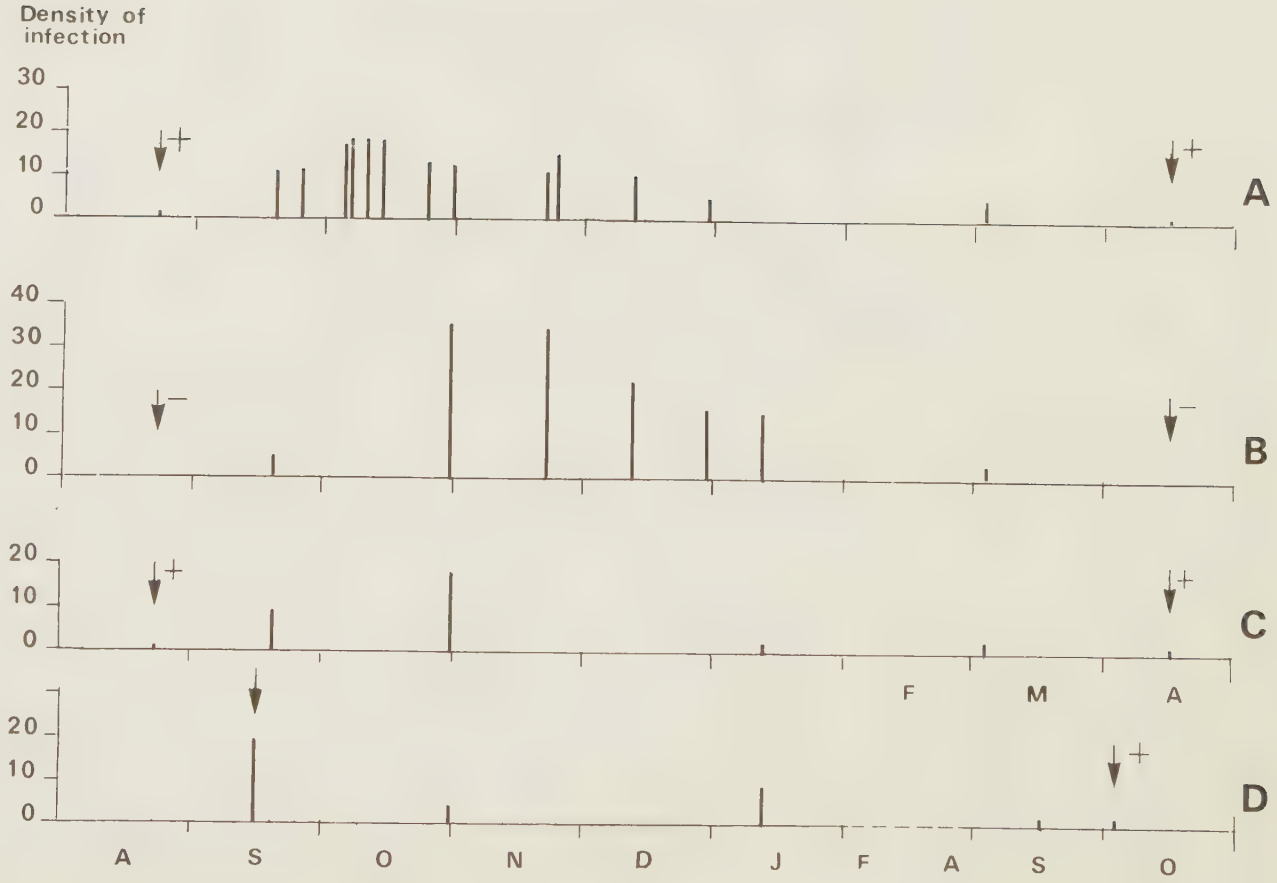
FIG.1


Density of infection
30
20
10
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A
40
30
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10
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B
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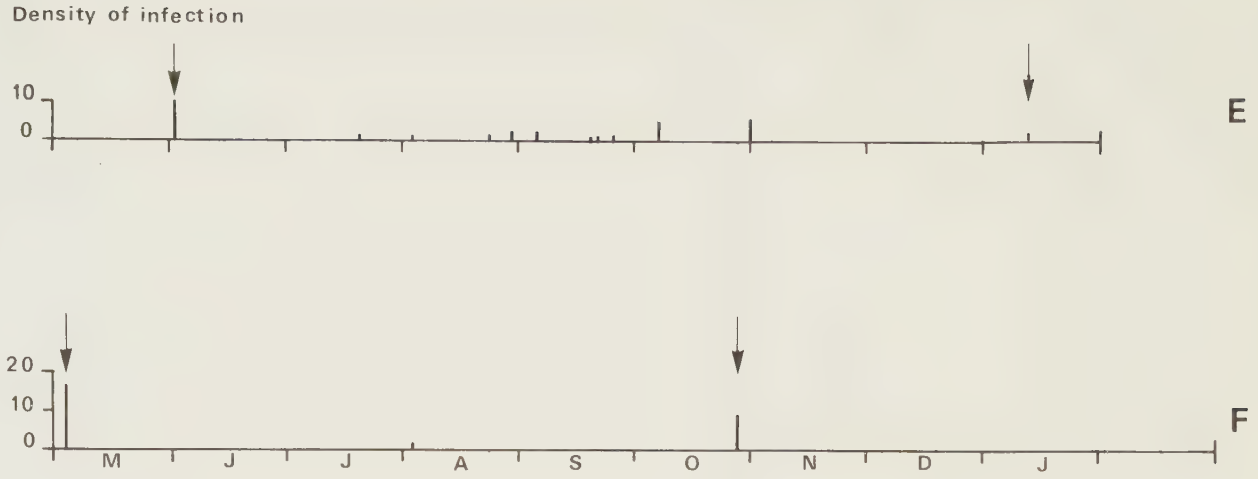

Fig 2. A-F. The course of the Karyolysus infection in Lacerta agilis. A, H_2, L. agilis, ad.♀ from Degeberga. B, H_3, L. agilis, juv.♂ from Degeberge. C, H_1, L. agilis, ad.♂ from Degeberga. D, B_{11}, L. agilis, ad.♂ from Maglehem. E, B_{10}, L. agilis, ad.♀ from Maglehem. F, B_7, L. agilis, ad.♂ from Maglehem.

A new Piroplasm *Sauroplasma boreale* sp. nov. (Protozoa, Haemosporidia) from the Sand Lizard *Lacerta agilis* L.

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ABSTRACT

The piroplasm Sauroplasma boreale sp. nov. (Protozoa, Haemosporidia), from the red cells of the lizard Lacerta agilis L., collected from Scania, southern Sweden and Mön, Denmark is described. 154 lizards from 21 localities in southern Sweden and Denmark were examined. Thirty-two of 52 lizards from 2 localities were found to be infected. A reptile piroplasm is described for the first time in Scandinavia.

INTRODUCTION

The genus Sauroplasma du Toit, 1937 parasitizing the red cells of lizards, is characterized by small, round parasites without any pigment. The typical form is that of a ring with a large vacuole. Only two species have been reported, viz. S. thomasi du Toit, 1937 from the lizard Zonurus (Cordylus) giganteus Smith and S. zonorum Pienaar, 1962 from Cordylus vittifer Reichenow, both from South Africa. Zakharyan (1970) found a Sauroplasma sp. in a lizard Gymnodactylus russowi Strauch. in the Fergana Valley, Uzbekistan. Uilenberg and Blanc (1966) found a Sauroplasma sp. from a Gecko Uroplatus fimbriatus Schneider from Madagascar.

There are no previous records of piroplasms from Scandinavian reptiles. In searching for blood parasites 154 Sand Lizards Laceraa agilis L. from 21 localities in Southern Sweden and Denmark were examined. Thirty-two of 52 from 2 localities were positive for a piroplasm. The present communication descbribes a new species of piroplasm, Sauroplasma boreale from L. agilis.

MATERIALS AND METHODS

Thin blood films from the tail tip were air-dried and fixed in methyl alcohol. The films were stained with Giemsa´s stain. Internal organs of lizards to be sectioned were fixed in Helly´s solution and cut in 4-5 μm sections by the usual paraffin method. The stains used were Delafield´s haematoxylin and Ehrlich´s haematoxylin-eosin.

DESCRIPTION OF SAUROPLASMA BOREALE SP. NOV.

Host: Lacerta agilis L.

Localities: North shore of Lake Snogeholmssjön in Scania, southern Sweden and Jydelejet on the island of Mön, Denmark.

Incidence: At Lake Snogeholmssjön 32 of 48 (67 %) and at Jydelejet 2 of 4 (50 %) examined lizards were infected.

Infection density: The infection was very light. In the specimen with the heaviest infection a count of 2800 cells showed an infection of 2 %. Very rarely two parasites were present in a single erythrocyte. In a few cases erythrocytes infected by both a piroplasm and a parasite of the genus Karyolysus Labbé were observed.

Structure: The parasites were small, normally ring-shaped bodies in the erythrocytes (Figs. 1-13, 16). Some were more irregular. In most of the parasites the outer membrane that formed the ring was clearly limited, partly by well-stained cytoplasm, partly by nuclear material. The outer membrane was reddish-purple. The central portion of the parasites was mostly clearly stained and in some cases there seemed to be an actual vacuole. Some parasites appeared as dark, round bodies without a clear part of cytoplasm. No granules of pigment could be observed in the cytoplasm.

The parasites revealed a variation in size and shape. Following forms were noted:

1. Small anaplasmoid bodies consisting of a chromatin granule (Figs. 10-11, 16A). They measured 0.8 to 1.2 μm in diameter.

2. Small ring-shaped bodies with a very thin outer membrane. Measurements 1.2 μm in diameter (Fig. 16B).

3. Piroplasms of "signet ring" type with central vacuole and a peripheral chromatin dot (Figs. 2, 16G).

4. Ring-shaped piroplasms with a dark cytoplasm without vacuole. Measurements 2.3 to 2.7 μm in diameter (Figs. 3, 9, 12, 16C).

5. Ring-shaped or irregular bodies of different size varying between forms with a large clear central portion and forms with a very small centrally of periferally situated vacuole (Figs. 1, 3-7, 10-13, 16D-F). They were

the most common parasites. The size varied from 3.5 × 3.8 μm to 1.5 × 1.5 μm. Mean of 30 parasites measured was 2.3 × 1.9 μm.

6. Rod-like bodies with a chromatin dot at the one end (Fig. 16H).

7. Forms of "budding-type" with two sometimes very small chromatin dots (Figs. 2, 8, 10-11, 16I-J). Nothing points to the budding being an actual process of multiplication. It rather seemed to be a variation of the nuclear part of the parasite. Multiplication of "binary fission" type was not observed. Stages similar to those referred to as "binary fission" by du Toit and Pienaar were double infections of the blood cells (Fig. 7).

The host cells retained normal size and stained normally and the host cell nucleus had its normal position and size. Schizogony was never observed in the erythrocytes.

DEVELOPMENT AND PATHOLOGY OF S. BOREALE IN LIZARDS

Examination of sections of liver and lung of infected lizards showed no stages of piroplasms. Blood from infected lizards showed intensely-staining granular accumulation in leucocytes (Figs. 14-15). The infected animals appeared to be healthy.

DISCUSSION

Du Toit (1937) placed his Sauroplasma thomasi into the new genus Sauroplasma, on the basis of general structure. According to him, Sauroplasma could be defined as "small, round or irregularly shaped unpigmented parasites of the red corpuscles of lizards. The typical form is that of a ring or signet ring with a large vacuole". Du Toit continues: "Multiplication takes place by binary fission or by a process of budding".

Piennar (1962) described Sauroplasma zonorum from a lizard Cordylus vittifer Reichenow in South Africa but mentions that it may be a morphological variation of S. thomasi.

The structural features of the parasie described above justify its inclusion in the genus Sauroplasma du Toit, 1937. It differs from S. thomasi in the outer membrane that formed the ring usually being thicker, in the dimensions of parasites, in the light infection, in host animal and in geographical distribution. The largest forms of S. thomasi measured 4.5 μm in length or diameter, while those of S. boreale measured 3.8 μm S. thomasi occurs in South Africa while the new parasite lives in northern Europe. It is therefore named Sauroplasma boreale.

Some facts point to the tick Ixodes ricinus L. being the transmitter.

Type: Slide marked Sauroplasma boreale from L. agilis in the Zoological Museum, University of Lund.

Acknowledgements - I am grateful to Professor Per Brinck, Head of the Department of Animal Ecology, Lund for providing laboratory facilities.

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Figs. 1-13. A series of microphotographs of Sauroplasma boreale illustrating the variation of parasites. Giemsa.

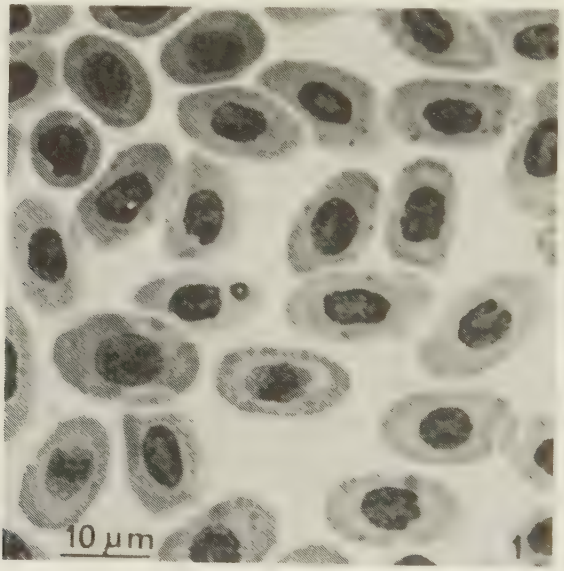


Fig 1. Typical ring-shaped piroplasm with clear central vacuole.

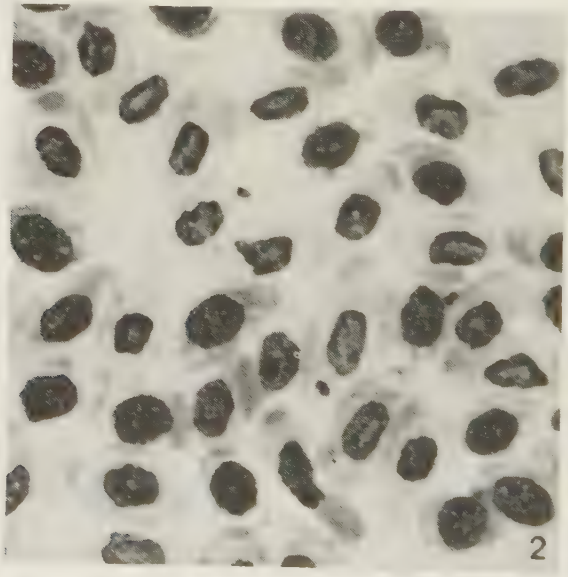


Fig 2. "Signet ring" type and "budding type".

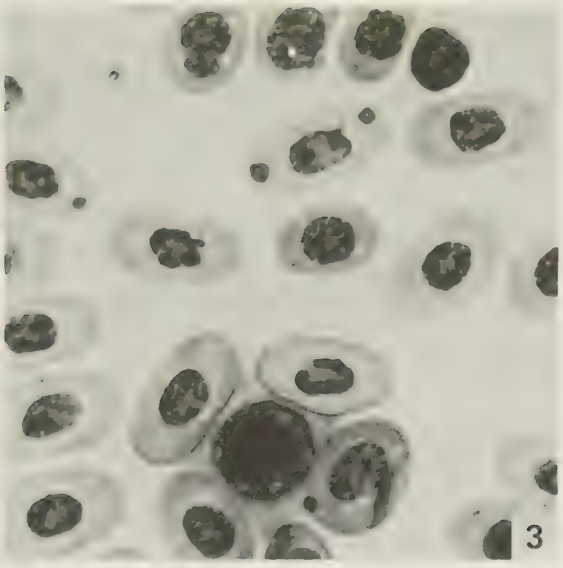
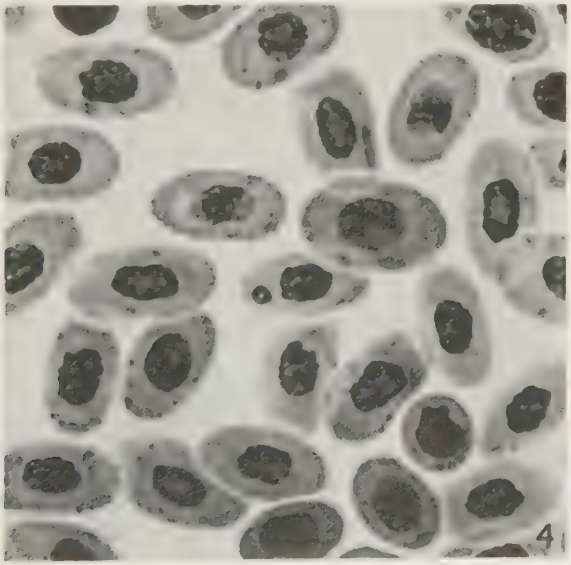
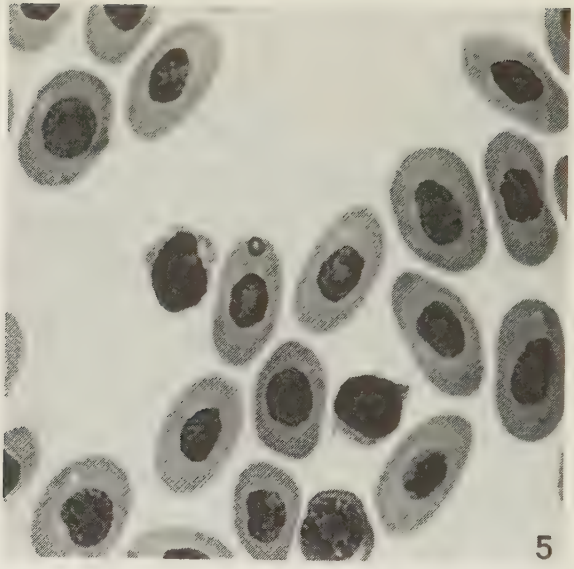


Fig 3. Double infection. One piroplasm without vacuole.



Figs. 4-7. Ring-shaped piroplasms. Note double infection in fig 7.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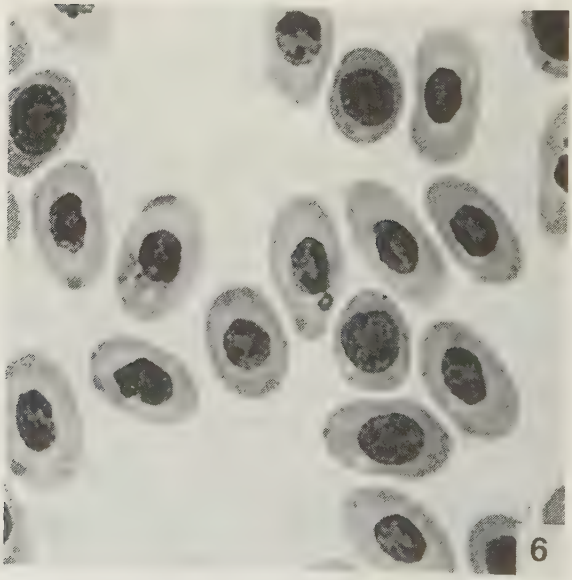
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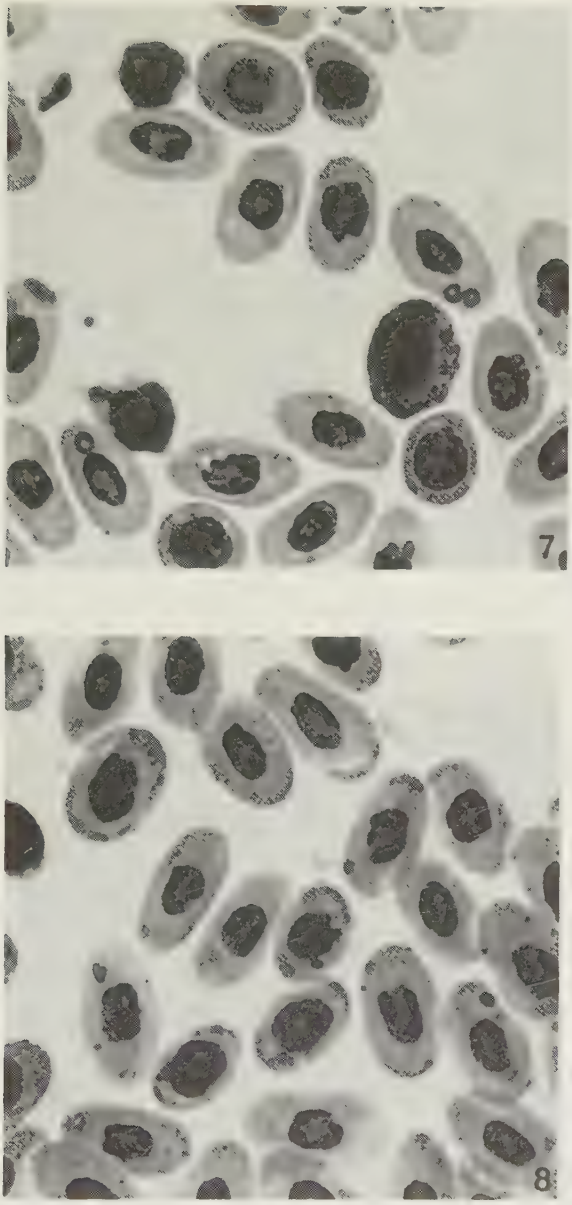


Fig 8. Parasite of "budding type".

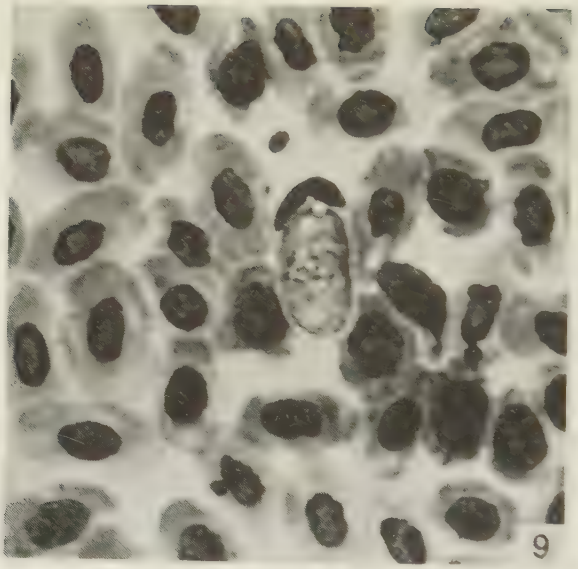
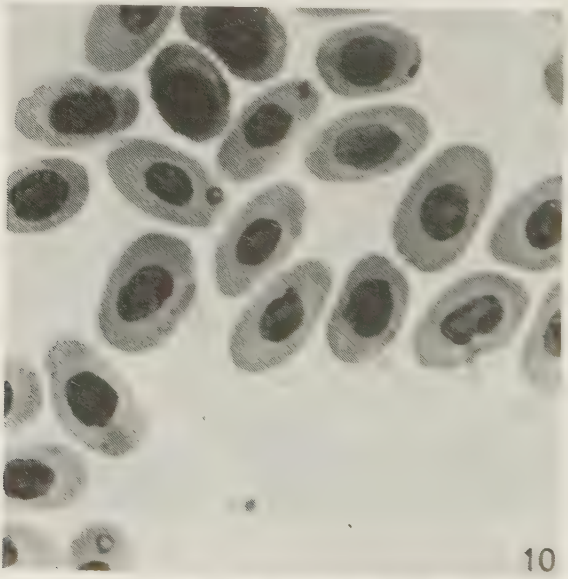
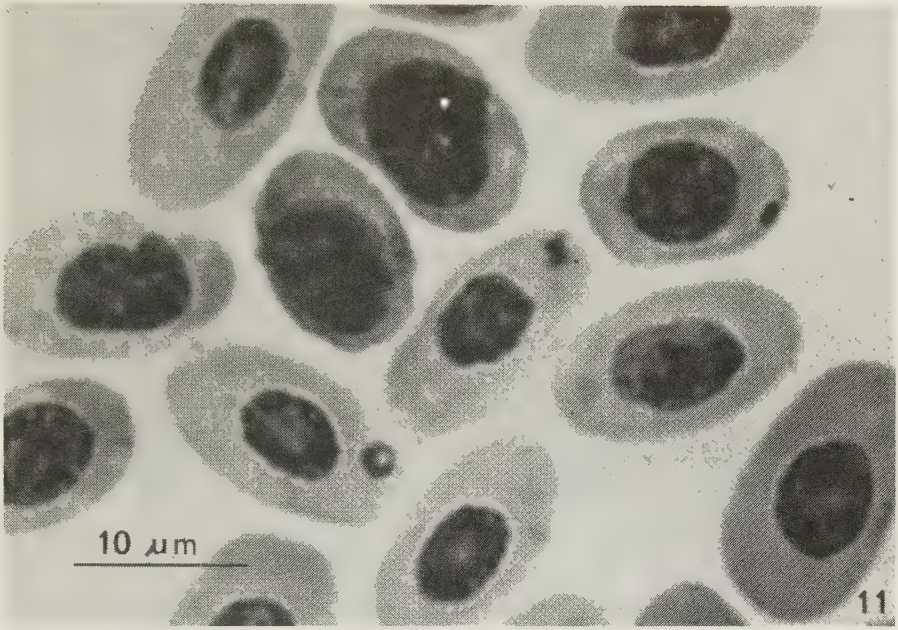


Fig 9. Blood cell infected by a piroplasm without vacuole and a parasite of the genus Karyolysus.



Figs. 10-11. Different stages of piroplasm: Ring-shaped parasites with small and with large vacuole, anaplasmoid body, and parasite of "budding type".


10 µm
11

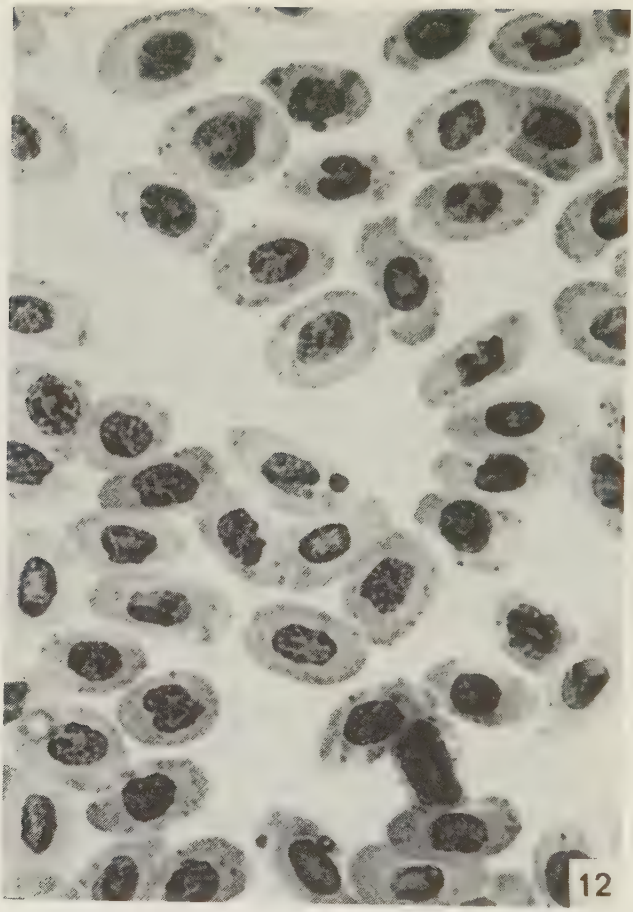


Fig 12. Prioplasms of two types.

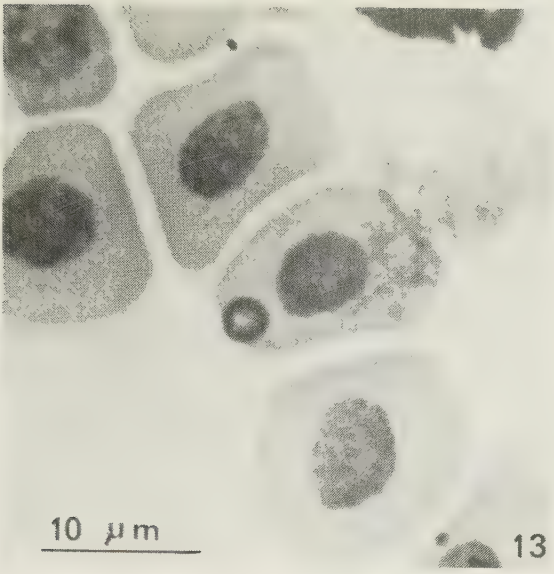
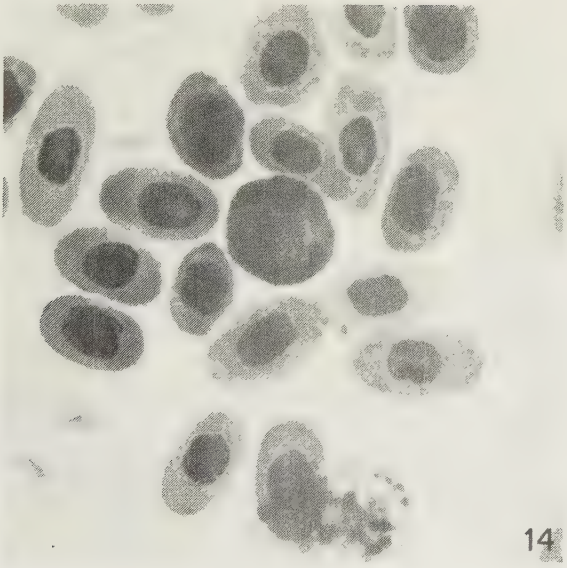
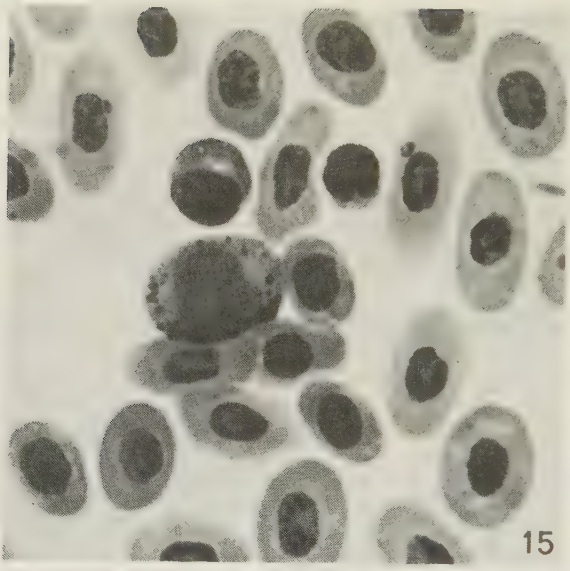


Fig 13. Typical ring-shaped parasite.



Figs 14-15. Granular accumulation in leucocytes. Giemsa.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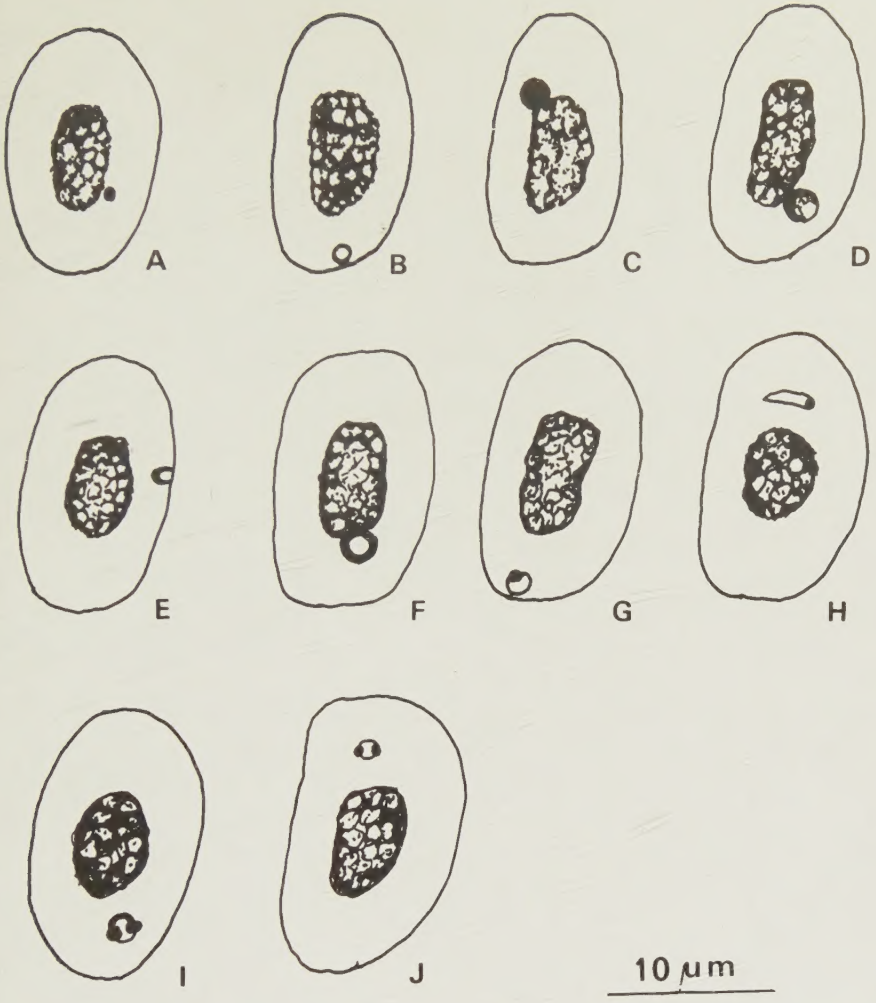

Fig 16. S. boreale. A, anaplasmoid body. B, small ring-shaped body. C, parasite with dark cytoplasm without vacuole. D-F, ring-shaped bodies of varying type. G, "signet ring" type. H, rod-like, modified "signet ring" type. I-J, piroplasms of "budding type".

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